ISSN 0254-4725

Fats and oils in human nutrition

Report of a joint expert consultation

FAO
FOOD AND
NUTRITION
PAPER

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Organized by the
Food and Agriculture Organization of the United Nations
and the
World Health Organization
Rome, 19-26 October 1993

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FIAT PANIS

Rome, 1994

Reprinted 1998

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M-80
ISBN 92-5-103621-7

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Acknowledgements

FAO and WHO express their gratitude for the contributions of the experts who participated in this consultation. A number of the participating experts prepared draft papers which, after discussion and amendment, formed the basis for the text of the chapters of this report. FAO and WHO also wish to thank the many other experts who contributed papers, and reviewed and modified the draft papers prior to the consultation. Special appreciation is offered to Dr Mark Hegsted, who served as Chairman of the Expert Consultation and provided guidance in the preparation of the report. The efforts of Dr Joyce Beare-Rogers, who undertook the large task of assisting the two organizations in preparing for the consultation, compiling and reviewing draft papers, and developing the final report are gratefully acknowledged.

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Chapter 1

Introduction

The role of dietary fats and oils in human nutrition is one of the most important areas of concern and investigation in the field of nutritional science. The findings of these investigations have wide-ranging implications for consumers, health-care providers and nutrition educators, as well as food producers, processors and distributors. New evidence concerning the benefits and risks associated with particular aspects of dietary fat is constantly emerging in both the scientific literature and the popular media. At times, controversies about these findings evolve. Sifting through all the claims and counterclaims, incomplete and incompatible studies, biases and competing interests, for the elements of truth and a prudent course of action is a challenge. Yet, this task is essential because changing views about the effects of dietary fats and oils can profoundly influence the consumption of various foods and, ultimately, health and nutritional status, agricultural production, food processing technologies, food marketing practices and nutrition education.

At the invitation of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), an international group of experts in nutrition, public health, food science and technology gathered in Rome from 19-26 October 1993 to consider the latest scientific evidence about dietary fats and oils. The experts attending the consultation discussed the many crucial and varied roles that dietary fats and oils play in human nutrition. They considered the intakes of different types and levels of dietary fats and oils and their associated health effects. They reviewed many of the technical factors associated with the production, processing, marketing and utilization of fats and oils. Finally, a series of recommendations about dietary fats and oils were made to assist policy makers, health-care specialists, the food industry, and consumers. This "Joint FAO/WHO expert consultation on fats and oils in human nutrition" was part of a continuing series of meetings on nutrition-related topics which are sponsored by FAO and WHO. This consultation was the second such meeting to have been held on fats and oils; the first was held in 1977.

This report of the meeting includes a discussion of the issues and evidence considered, the conclusions and recommendations of the group and a bibliography. A wide range of topics was reviewed by the experts and this is reflected in the report. This report includes chapters on the following topics: the composition of dietary fat; aspects of fat digestion and metabolism; global trends in the availability of edible fats and oils; processing and refining edible oils; selected uses of fats and oils in food; lipids in early development; health, obesity and energy values; coronary heart disease and lipoproteins; isomeric fatty acids; cancer and dietary fat; dietary fat and immune response; dietary fat, hypertension and stroke; non-glyceride components of fats; and nutrition labelling.

Since efforts to address one aspect of diet-health relationships can affect other aspects as well, care needs to be taken not to over-emphasize any single issue to the detriment of others. The recommendations, therefore, reflect a synthesis and weighing of various concerns. It should be noted that the evidence related to the different topics varies considerably. Until more scientific information accumulates and the understanding of the complex metabolic interactions that determine nutritional and health status increases, it will not be possible to reach full agreement on each topic. This is a dilemma which is reflected in the nature of the conclusions and recommendations that emerged from the consultation. The final conclusions and recommendations are provided in this chapter, preceded by a brief note identifying key issues. We encourage readers to examine the chapters of the report for more detailed information about the topics considered and for insights into the deliberations leading to the general conclusions and recommendations of the consultation.

General conclusions and recommendations of the consultation

Minimum desirable intakes of fats and oils

Adults. Adequate amounts of dietary fat are essential for health. In addition to their contribution to meeting energy needs, intakes of dietary fat must be sufficient to meet requirements for essential fatty acids and fat soluble vitamins. The minimum intake consistent with health varies throughout a person's life and among individuals. Adequate intake of dietary fat is particularly important prior to and during pregnancy and lactation. Increasing the availability and consumption of dietary fats is often a priority for overcoming the problems of protein-energy malnutrition. Recommendations to populations concerning desirable ranges of fat intakes may vary according to prevailing conditions, especially dietary patterns and the prevalence of diet-related noncommunicable diseases.

Recommendations on minimum intakes of adults:

- For most adults, dietary fat should supply at least 15 percent of their energy intake.
- Women of reproductive age should consume at least 20 percent of their energy from fat.
- Concerted efforts should be made to ensure adequate consumption of dietary fat among populations where less than 15 percent of the dietary energy supply is from fat.

Infants and young children. Both the amount and quality of dietary fat consumed can affect child growth and development. These influences are mediated through energy levels and through the action of specific fatty acids and various non-glyceride components of the fat. Breast-milk provides between 50-60 percent energy as fat, and during the weaning period (that is, the transition from full breast-feeding to no breast-feeding), care needs to be taken to prevent dietary fat intakes from falling too rapidly or below the required levels. The use of fat, especially vegetable oils, in the foods fed to weanling infants and young children is an effective way to maintain the energy density of their diets.

The consumption of adequate amounts of essential acids is also important for normal growth and development. Arachidonic acid and docosahexaenoic acid (DHA) are particularly important for brain development, and breast-milk is a good source of these fatty acids.

Particular problems exist for preterm infants who had an insufficient intra-uterine supply of arachidonic acid and DHA and who were born with low fat reserves.

Recommendations regarding infant and young child feeding:

- Infants should be fed breast-milk if at all possible.
- The fatty acid composition of infant formulas should correspond to the amount and proportion of fatty acids contained in breast-milk.
- During weaning and at least until two years of age, a child's diet should contain 30-40 percent of energy from fat and provide similar levels of essential fatty acids as are found in breast-milk.

Upper limits of fat/oil intakes

Excessive dietary fat intake has been linked to increased risk of obesity, coronary heart disease and certain types of cancer. The mechanisms by which these are linked are complex, varied and, in many instances, not clearly understood. Elevated levels of serum cholesterol and LDL constitute major risk factors for atherosclerosis and coronary heart disease. The degree of risk of these and other factors may vary according to, *inter alia:* type and level of fatty acid intakes, percentage of energy from total fat, dietary cholesterol, lipoprotein levels, intakes of antioxidants and dietary fibre, activity levels and health status. Low-fat diets are often lower in cholesterol and higher in antioxidants and dietary fibre. Among adults, there is no nutritional advantage to consuming high-fat diets once essential energy and nutrient needs are met.

Recommendations on upper limits of dietary intakes:

- Active individuals who are in energy balance may consume up to 35 percent of their total energy intake from dietary fat if their intake of essential fatty acids and other nutrients is adequate and the level of saturated fatty acids does not exceed 10 percent of the energy they consume.
- Sedentary individuals should not consume more than 30 percent of their energy from fat, particularly if it is high in saturated fatty acids which are derived primarily from animal sources.

Saturated and unsaturated fatty acids, and cholesterol

The saturated fatty acids - lauric, myristic and palmitic - elevate serum cholesterol and low density lipoprotein (LDL) levels. Stearic acid does not elevate serum cholesterol or LDL levels, however, other health effects are, as yet, undefined. Polyunsaturated linoleic acid moderately reduces serum cholesterol and LDL levels. Monounsaturated oleic acid appears to be neutral in regard to LDL, but raises high density lipoproteins (HDL) modestly. Dietary cholesterol elevates serum cholesterol and LDL levels, but the extent of the increase is highly variable.

Recommendations on intakes of saturated and unsaturated fatty acids:

- Intakes of saturated fatty acids should provide no more than 10 percent of energy.
- Desirable intakes of linoleic acid should provide between 4 and 10 percent of energy. Intakes in the upper end of this range are recommended when intakes of saturated fatty acids and cholesterol are relatively high.
- Reasonable restriction of dietary cholesterol (less than 300 mg/day) is advised.

Isomeric fatty acids

Unsaturated vegetable oils are frequently partially hydrogenated to produce more solid, plastic or stable fats. During this process, an assortment of *cis* and *trans* isomers is formed. Compared to oleic acid, the *trans* isomers in partially hydrogenated vegetable oils tend to elevate serum LDL levels and may lower HDL levels. High intakes of *trans* fatty acids are undesirable, but it is, as yet, uncertain whether the use of *trans* or saturated fatty acids is preferable where such fatty acids are required to formulate food products.

Recommendations concerning isomeric fatty acids:

- Consumers should substitute liquid oils and soft fats (that is, those which are soft at room temperature) for hard fats (more solid at room temperature) to reduce both saturated fatty acids and *trans* isomers of unsaturated fatty acids.
- Food manufacturers should reduce the levels of *trans* isomers of fatty acids arising from hydrogenation.
- Governments should monitor the levels of isomeric fatty acids in the food supply.
- Governments should limit the claims concerning the saturated fatty acid content of foods which contain appreciable amounts of *trans* fatty acids, and should not allow foods that are high in *trans* fatty acids to be labelled as being low in saturated fatty acids.

Substances associated with fats and oils

Substantial evidence indicates that relatively high intakes of fruits and vegetables - sources of various antioxidants, carotenoids and other non-glyceride components - reduce the risk of coronary heart disease and some cancers. Yet, specific conclusions and recommendations concerning the general health benefits and desirable intakes of these substances cannot be made on the basis of current evidence.

Processing and refining techniques used to eliminate or reduce negative characteristics of edible oils can also lead to the loss of various nutritionally beneficial components such as antioxidants and carotenoids. However, producers can minimize such losses through appropriate processing, refining and storage techniques and they are encouraged to do so.

Recommendations on antioxidants and carotenoids:

- In countries where vitamin A deficiency is a public health problem, the use of red palm oil, wherever readily or potentially available, should be encouraged. If the oil is refined, processing techniques that preserve the carotenoid and tocopherol content of red palm oil should be utilized.
- Tocopherol levels in edible oils need to be adequate to stabilize the unsaturated fatty acids present. Therefore, foods high in polyunsaturates should contain at least 0.6 mg tocopherol equivalents per gram of polyunsaturated fatty acid. Higher levels may be necessary for fats that are rich in fatty acids containing more than two double bonds.

Essential fatty acids

The n-6 and n-3 fatty acids have critical roles in the membrane structure and as precursors of eicosanoids, which are potent and highly reactive compounds. Various eicosanoids have widely divergent, and often opposing effects on, for example, smooth muscle cells, platelet aggregation, vascular parameters (permeability, contractility), and on the inflammatory processes and the immune system. Since they compete for the same enzymes and have different biological roles, the balance between the n-6 and the n-3 fatty acids in the diet can be of considerable importance.

A number of studies have shown that the consumption of foods (such as oil-rich fish) containing the long-chain n-3 fatty acids, eicosapentaenoic acid (EPA) and DHA, is associated with decreased risk of coronary heart disease (CHD), probably because of mechanisms not related to serum lipoprotein levels.

Essential fatty acids are especially important for normal fetal and infant growth and development, in particular, for brain development and visual acuity. In well-nourished women, approximately 2.2 grams of essential fatty acids are deposited in maternal and fetal tissues each day throughout pregnancy.

Recommendations concerning essential fatty acid intakes:

- The ratio of linoleic to α-linolenic acid in the diet should be between 5:1 and 10:1.

- Individuals with a ratio in excess of 10:1 should be encouraged to consume more n-3 rich foods such as green leafy vegetables, legumes, fish and other seafood.
- Particular attention must be paid to promoting adequate maternal intakes of essential fatty acids throughout pregnancy and lactation to meet the requirements of fetal and infant development.

Scientific and programmatic needs

Adequate information on nutritional status, dietary intakes and the composition of foods is required for designing and monitoring programmes to improve nutrition, including the promotion of appropriate intakes of dietary fats and oils.

Governments and health authorities in all countries need to be aware of the escalating risk of non-communicable diseases that follow the adoption of inappropriate dietary practices and less active life-styles.

Recommendations on dietary information and programme needs:

- Standard methods and reference materials should be used in the analysis of the fatty acid content of foods and in the preparation of nutrient databases.
- Adequate food composition data on fats should be widely available and accessible with each food item being identified by unambiguous descriptive factors.
- The standard Atwater factor of 9.0 kilocalories (37.7 KJ) per gram of fat should be used for calculating the energy value of fat in all nutrition surveys and food composition tables.
- Periodic surveys of the weight status (body mass index) of adults are desirable in all countries to help identify trends and populations affected by or at greater risk of undernutrition and diet-related non-communicable diseases and to monitor the impact of interventions.

Chapter 2

Composition of dietary fat

Dietary fat includes all of the lipids in plant and animal tissue which are eaten as food. The most common fats (solid) or oils (liquid) are a mixture of triacylglycerols (triglyceride) with minor amounts of other lipids. The fatty acids present in various lipid molecules are the moieties of great nutritional interest.

Fatty acids

The most abundant fatty acids have straight-chains of an even number of carbon atoms. There is a wide spectrum of chain-lengths, ranging from a four-carbon fatty acid in milk to thirty-carbon fatty acids in some fish oils. Frequently, the fatty acids have eighteen carbons. Double bonds along the carbon chain or substituents on it are designated chemically by counting the carboxyl carbon as position 1. Thus, the double bonds in linoleic acid give it the chemical systematic name of 9, 12-octadecadienoic acid. A short-hand abbreviation for linoleic acid is 18:2 (eighteen carbon atoms: two double bonds). Its last double bond is six carbons from the methyl end, an important feature for some enzymes. This is considered a n-6 or $\omega 6$ fatty acid (Figure 2.1). The n-6 nomenclature is used in this report.

The common (trivial) name, the chemical systematic name and the short-hand abbreviation for some dietary fatty acids are provided in Table 2.1. The double bonds in the fatty acids are in the *cis*-configuration. The first member of the n-6 series of fatty acids is linoleic acid and the first member of the n-3 series is α-linolenic acid (9, 12, 15-octadecatrienoic acid). The n-6 and n-3 polyunsaturated fatty acids have *cis* double bonds that are interrupted by methylene groups. A double bond may change from a *cis* to a *trans* configuration (geometric isomerization) or move to another position along the carbon chain (positional isomerization) as illustrated in Figure 2.2. The shape of a *trans* fatty acid is similar to that of a saturated fatty acid. As a result, *trans* fatty acids have a higher melting point than their *cis* isomer. The *trans* isomer may be regarded as an intermediate between an original *cis* unsaturated fatty acid and a completely saturated fatty acid.

Acylglycerides. The type of fatty acid and the position in which it is esterified to glycerol determine the characteristics of acylglycerides. In addition to glycerides which have three esterified fatty acids, diacylglycerides (diglycerides) and monoacylglycerides (monoglycerides) occur in raw food or food ingredients (Figure 2.3).

FIGURE 2.1
Diagram of fatty acids

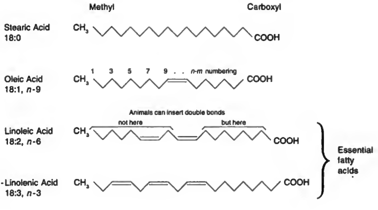
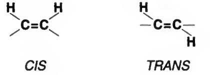


FIGURE 2.2
Structure of *cis* and *trans* double bonds



There is some specificity in the position occupied by the fatty acids. Animal depot fats tend to have a saturated fatty acid in position 1 and an unsaturated fatty acid in position 2. Fatty acids in position 3 appear to be more randomly distributed, although polyunsaturated fatty acids often accumulate there.

Phospholipids. Phospholipids are components of membranes which occur in foods and extracted oils. The general structure of phosphoglycerides is shown in Figure 2.4.

A saturated fatty acid is usually esterified at position 1 and a polyunsaturated fatty acid at position 2. The polar groups which contain phosphorus and an organic base provide the lipid molecule with a hydrophilic region. In addition to phosphoglycerides, phospholipids include sphingomyelins and cerebroside which are based on sphingosine rather than glycerol. Although phospholipids constitute only a small fraction of total dietary fat, they can be an important source of essential fatty acids.

TABLE 2.1
Some dietary fatty acids

Common name	Systematic name	Abbreviation	Fatty acid family
capric	decanoic	10:0	
lauric	dodecanoic	12:0	
myristic	tetradecanoic	14:0	
palmitic	hexadecanoic	16:0	
stearic	octadecanoic	18:0	
arachidic	eicosanoic	20:0	
behenic	docosanoic	22:0	
lignoceric	tetracosanoic	24:0	
palmitoleic	9-hexadecenoic	16:1	n-7
oleic	9-octadecenoic	18:1	n-9
gadoleic	11-eicosaenoic	20:1	n-9
cetoleic	11-docasaenoic	22:1	n-11
erucic	13-docasaenoic	22:1	n-9
nervonic	15-tetracosaenoic	24:1	n-9
linoleic	9,12-octadecadienoic	18:2	n-6
α-linolenic	9,12,15-octadecatrienoic	18:3	n-3
γ-linolenic	6,9,12-octadecatrienoic	18:3	n-6
dihomo-γ-linolenic	8,11,14-eicosatrienoic	20:3	n-6
	5,8,11-eicosatrienoic	20:3	n-9
arachidonic	5,8,11,14-eicosatetraenoic	20:4	n-6
EPA	5,8,11,14,17-eicosapentaenoic	20:5	n-3
adrenic	7,10,13,16-docosatetraenoic	22:4	n-6
	7,10,13,16,19-docosapentaenoic	22:5	n-3
DPA	4,7,10,13,16-docosapentaenoic	22:5	n-6
DHA	4,7,10,13,16,19-docosahexaenoic	22:6	n-3

FIGURE 2.3
Diagram of acylglycerides

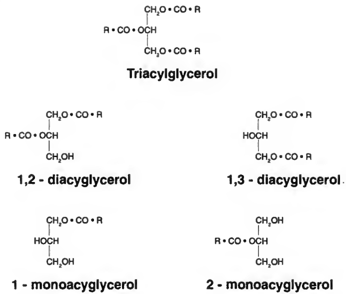
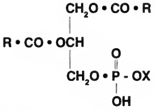


FIGURE 2.4
Diagram of phospholipids



Phospholipid where X is choline, ethanolamine, serine, inositol, glycerol.

Non-glyceride components

The growing realization of the importance of the non-glyceride components, sometimes called "minor constituents", in fats and oils warranted inclusion of this topic in the expert consultation. Non-glyceride components are minor constituents only in terms of their concentrations relative to triacylglycerols. New information about these constituents of fat stems from improvements in the ability to analyze them and studies of their properties.

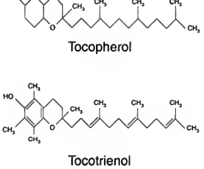
Vitamin E. Vitamin E consists of a mixture of lipid-soluble phenols characterized by an aromatic chromanol head and a side chain of 16-carbon atoms. The tocopherols have a saturated hydrocarbon tail, whereas the tocotrienols are the farnesylated analogues having an unsaturated isoprenoid tail. The number and position of the methyl groups on the chromanol ring give rise to the different α-, ß-, γ- and δ-tocopherol and tocotrienol isomers (Figure 2.5).

Vegetable oils and the products made from them usually contain large amounts of tocopherol, especially the α, ß and γ isomers. In addition, certain vegetable oils, particularly palm oil (Qureshi *et al.*, 1991a) and rice bran oil (Rogers *et al.*, 1993), are rich sources of tocotrienols which have weak vitamin E activity but act as antioxidants and provide stability against oxidation.

Carotenoids. Carotenoids are highly unsaturated polyisoprene hydrocarbons that are lipid soluble. Over 75 different carotenoids are known to occur in animal fats and vegetable oils. The most common are α, ß and γ carotene, lycopene, lutein and xanthopylls (Figure 2.6). The carotenoids and their derivatives are generally responsible for the yellow to deep-red colour of fruits, vegetables, cereals and crude palm oil. Carotenoids are the precursors of vitamin A, with ß-carotene having the highest provitamin A activity.

Vitamins A and D. A traditional source of vitamin A is butterfat. Fish oils are a common source of vitamin D. Margarines, which are fortified with vitamins A and D by law in most countries, also make an important contribution to ensuring adequate intakes of these nutrients.

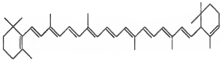
FIGURE 2.5
Diagram of tocopherol and tocotrienol



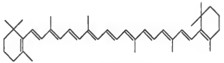
Tocopherol

Tocotrienol

FIGURE 2.6
Diagram of α - carotene and β - carotene



α - carotene



β - carotene

Other components

Sterols. Cholesterol is the principal sterol of animal products. The major sterols of plants are β-sitosterol, campesterol and stigmasterol, although several others are known to exist (Formo *et al.*, 1979); the side chains of cholesterol and several plant sterols are shown in Figure 2.7. They occur in the free form or are esterified to such compounds as fatty acids, glucosides or ferulic acid (oryzanol). The sterol content of dietary fats and oils ranges from 0.01 to 2 percent (Itoh, Tamura and Matsumoto, 1973a).

Methylsterols and triterpene alcohols. Sterols methylated at the 4-OH position occur in common vegetable oils at concentrations of 0.01 to 0.4 percent with rice bran oil and sesame oil having the highest levels (Itoh, Tamura and Matsumoto, 1973b). Corresponding concentrations of triterpene alcohols, comprised of five condensed cyclohexane rings, are 0.01 to 1.2 percent. Rice bran oil is the only one at the upper level.

Squalene. The predominant hydrocarbon in dietary fat is squalene. It is an intermediate in the synthesis of sterol from acetate, and it is found in particularly high quantities in some fish and olive oil. The concentration in most vegetable oils is below 30 mg/100 g (Formo *et al.*, 1979).

Oryzanols. Oryzanols are compounds consisting of ferulic acid esterified to a variety of plant sterols and triterpene alcohols (Figure 2.8). Although large amounts are found in crude rice bran and linseed oils, oryzanols are not widely distributed in other oils (*Ibid.*).

FIGURE 2.7
Side chains of cholesterol and several plant sterols

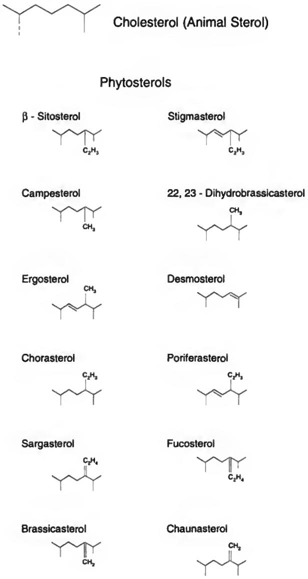
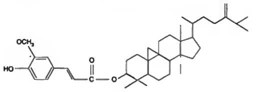


FIGURE 2.8
Chemical structure for oryzanol (24-methylene-cycloartanol ester of ferulic acid)



Chapter 3

Aspects of fat digestion and metabolism

Digestion, absorption and transport

Most dietary fat is supplied in the form of triacylglycerols which must be hydrolyzed to fatty acids and monoacylglycerols before they can be absorbed. In children and adults, fat digestion is efficient and is nearly completed in the small intestine. In the newborn, the pancreatic secretion of lipase is low. The digestion of fat in babies is augmented by the lipases secreted from the glands of the tongue (lingual lipase) and a lipase present in human milk. The stomach is part of the process of fat digestion because of its churning action which helps to create an emulsion. Fat entering the intestine is mixed with bile and is further emulsified. The emulsion is then acted upon by lipases secreted by the pancreas. Pancreatic lipase catalyzes the hydrolysis of fatty acids from positions 1 and 3 to yield 2-monoacylglycerols (Tso, 1985). Phospholipids are hydrolysed by phospholipase A_2 and the major products are lysophospholipids and free fatty acids (Borgstrom, 1974). Cholesterol esters are hydrolyzed by pancreatic cholesterol ester hydrolase.

The free fatty acids and monoglycerides are absorbed by the enterocytes of the intestinal wall. In general, fatty acids which have a chain length of less than 14 carbons enter directly into the portal vein system and are transported to the liver. Fatty acids with 14 or more carbons are re-esterified within the enterocyte and enter the circulation via the lymphatic route as chylomicrons. However, the portal route has been described as an absorptive route for dietary long chain fatty acids as well (McDonald *et al.*, 1980). Fat soluble vitamins (vitamins A, D, E and K) and cholesterol are delivered directly to the liver as part of the chylomicron remnants.

Diseases that impair the secretion of bile, such as biliary obstruction or liver diseases, lead to severe fat malabsorption, as do diseases that influence the secretion of lipase enzymes from the pancreas, such as cystic fibrosis. As a result, medium-chain triglycerides can be better tolerated in individuals with fat malabsorption and these are often used as a source of dietary energy. Complete absorption of lipids from the intestine may be marginally affected by a high amount of fibre in the diet. Fat absorption is illustrated in Figure 3.1.

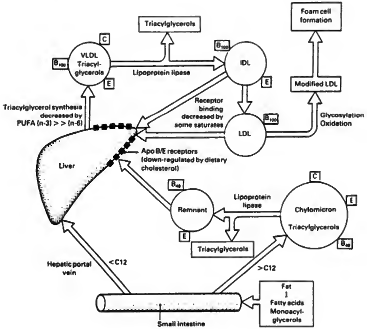
Fatty acids are transported in the blood as complexes with albumin or as esterified lipids in lipoproteins. These consist of a core of triacylglycerols and fatty acid esters of cholesterol, and a shell of a single layer of phospholipids interspersed with unesterified cholesterol. Coiled chains of one or more apolipoproteins extend over the surface and, with the amphipatic phospholipids, enable the lipids in the core to be carried in the blood. They also regulate the reaction of the lipid package with specific enzymes or bind the particle to cell surface receptors.

Chylomicrons are lipoprotein particles derived from dietary fat and packaged by the mucosa cells. They enter the blood stream via the lymph vessels. Lipoprotein lipase located on the interior walls of the capillary blood vessels hydrolyses the triacylglycerols, releasing fatty acids. These enter the adipose tissue where they are stored, and the muscles where they serve as fuel. The remnants of chylomicrons are cleared by the liver within a few hours of the ingestion of a fat-containing meal.

Very-low density lipoproteins (VLDL) are large triacylglcerol-rich particles produced in the liver from endogenous fat, as opposed to chylomicrons which transport exogenous fat. VLDLs are the main carriers of triacylglycerols which are also processed by lipoprotein lipase and supply fatty acids to adipose and muscle tissues.

Low-density lipoproteins (LDL) are the end products of VLDL metabolism. The core consists mainly of cholesterol esters and its surface has only one type of apolipoprotein,

FIGURE 3.1
The influence of dietary fat on lipoprotein metabolism



Source: Reproduced with permission from the British Nutrition Foundation, 1992 apoB. About 60-80 percent of cholesterol in plasma is carried by LDLs. Average LDL values vary among populations because of genetic and environmental factors, however, diet is probably a major determinant of these values.

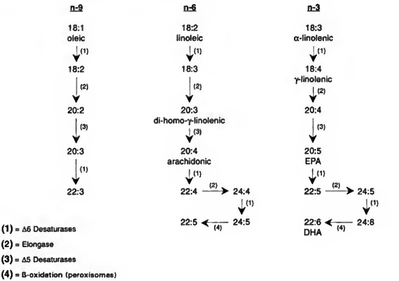
High-density lipoproteins (HDL) carry 15-40 percent of plasma cholesterol. They are probably formed in the circulation from precursors made in the liver and in the gut. The major apolipoprotein of HDL is apoA-1. In humans, LDL delivers cholesterol to the liver and HDL may transfer cholesterol to other lipoprotein particles. There is evidence that HDL actively protects vessel walls (NIH Consensus, 1993). It is not known whether manipulation of HDL levels by diet affects the development of atherosclerosis.

Lipoprotein(a) or Lp(a) is a complex of LDL with apolipoprotein(a). This apoprotein has a sequence homology with the pro-enzyme plasminogen which is involved with the dissolution of blood clots (Scanu and Scandiani, 1991). The concentration of Lp(a) is largely determined by genetics.

Metabolism of fatty acids

Saturated fatty acids and monounsaturated fatty acids can be biosynthesized from carbohydrates and proteins. Unsaturated fatty acids may be substrates for desaturases and elongases, as shown in the scheme for conversion in the n-9, n-6 and n-3 families of fatty acids (Figure 3.2).

FIGURE 3.2
Sequence of conversions of unsaturated fatty acids



The first members of each fatty acid family (oleic, linoleic and α-linolenic acid) compete for the same 6-desaturase, with rates of conversion increasing with the number of double bonds. This rate-limiting enzyme is under the control of many dietary and hormonal factors (Brenner, 1989; 1990) and is believed to be important in the synthesis of 22:6 n-3. Such an effect may explain why high intakes of linoleic acid reduce the level of 22:6 n-3. Similarly, the 5-desaturase is modulated by dietary and hormonal factors. The C_{20} and C_{22} members of the n-6 and n-3 families can inhibit desaturation earlier in the sequence of fatty acid conversion (Fisher, 1989).

It appears that the 4-desaturation does not involve another specific desaturase, but an elongation, then 6-desaturase, both microsomal processes, followed by retroconversion by the peroxisomal β-oxidation pathway (Voss *et al.*, 1991). The desaturation is thus a process based on intracellular cooperation. The nutritional significance of this additional role for 6-desaturase has yet to be evaluated. The activities of the elongases appear to be greater than those of desaturases (Sprecher, 1989).

Essential fatty acids

The essential fatty acids are those that must be supplied in the diet and they include members of both the n-6 and n-3 series. Under conditions of a dietary deficiency of linoleic acid, the most abundant unsaturated fatty acid in tissue, oleic, is desaturated and elongated to eicosatrienoic, n-9, which is normally found in trace amounts only. The accumulation of this fatty acid is considered to be a marker of essential fatty acid deficiency (Holman, 1970). When the diet has a low n-3 fatty acid content, compared to the n-6, there is a reduction of 22:6 n-3 associated with a compensatory accumulation of 22:5 n-6 in tissues (Galli, Agradi and Paoletti, 1974).

The most abundant product of the n-6 pathway is arachidonic acid found in glycerophospholipids where it is selectively esterified in the 2-position. Diets containing fats of animal origin provide appreciable amounts of preformed arachidonic acid.

The efficiency of the n-3 pathway in humans has been open to question. However, human studies on the conversion of deuterated linoleic and α-linolenic acids to their long chain derivatives showed that the efficiency for the conversion of linoleic to arachidonic was 2.3 percent, while the conversion of α-linolenic to its long chain derivatives was 18.5 percent (Emken *et al.*, 1992). These conversion values, measured as the accumulation of products in plasma, were obtained in subjects with relatively low intakes of linoleic acid (around 5 percent). When the diet contained around 9 percent linoleic acid, the conversions were reduced to 1 percent for linoleate and 11 percent for linolenate. These data do indicate that the synthesis of n-3 metabolites from 18:3 occurs in adults.

Fish oil, which is rich in eicosapentaenoic acid compared to linseed oil, greatly enhances the concentration of the long-chain derivatives in human blood (Sanders and Roshanai, 1983). High levels of eicosapentaenoic acid and docosapentaenoic acid in blood platelets or other cells are attained only when they are provided as such in the diet. The n-3 fatty acids, like the n-6 fatty acids, are incorporated into the 2-position of membrane phospholipids.

The polyunsaturated fatty acids for membrane phospholipids come from the diet or endogenous sources. The availability is influenced by deacylation-reacylation, particularly at position-2 of phospholipids (Lands *et al.*, 1982), the specificity of diacylglycerol kinases in phospholipid synthesis (McDonald *et al.*, 1988), and remodelling of donor and acceptor phospholipids. In all of these processes, competition exists between the fatty acids of the n-6 and n-3 series.

The essentiality of linoleic acid was demonstrated in studies of rats. When rats were fed a fat-free diet, they developed specific symptoms that were prevented by the provision of linoleic acid. Linoleic acid deficiency, a rare condition in humans, was described in children given fat-free diets (Hansen *et al.*, 1963). The children developed skin disorders similar to those produced in rats that were cured by the administration of linoleic acid. To prevent the accumulation of ecosatrienoic acid (20:3 n-9), the amount of linoleic acid required in the diet is at least 1 percent of the energy intake. This applies to all the animal species tested. Intakes between 4 and 10 percent of the energy are currently consumed by different population groups, and these appear to be compatible with optimal health status.

Linoleic acid deficiency may develop as a secondary condition in other disorders, such as protein-energy malnutrition and fat malabsorption, or as a consequence of total parenteral nutrition with inadequate linoleic acid intakes. Under conditions of adequate linoleic acid intake, the ratio of triene to tetraene is below 0.2 (Mohrhauer and Holman, 1963). A better assessment of linoleic acid deficiency can be obtained with a profile of all polyunsaturated fatty acids in serum (Holman and Johnson, 1981).

The n-3 fatty acid, docosahexaenoic acid (DHA, 22:6), is present in high concentrations in the central nervous system, in the cell membranes, and the visual system (Tinoco, 1982; Neuringer *et al.*, 1984; Bourre *et al.*, 1989). The role of DHA for optimal neuronal function and visual acuity is discussed in relation to early development later in this report.

The balance between n-6 and n-3 fatty acids in the diet is important because of their competitive nature and their essential and different biological roles. It is suggested that the relative amounts of linoleic and α-linolenic acids in the diet should be below 10:1.

Biological roles

Membrane structure. Unsaturated fatty acids in membrane lipids play an important role in maintaining fluidity (Lynch and Thompson, 1984). In the skin, linoleic acid plays a specific role where it is linked to some very long chain fatty acids (C30-C34) in the acyl ceramides. These form an intercellular matrix to maintain the epidermal permeability barrier (Hansen and Jensen, 1985).

In membranes, interactions between lipids and proteins may depend upon a specific polyunsaturated fatty acid. This appears to be the case for mammalian rod outer segments which are very rich in docosahexaenoic acid.

Three examples of lipid-protein interactions that control the metabolic functions of membranes were noted: first, the catalytic properties of transporter proteins; second, the activities of enzymes such as the Ca/Mg ATPase of sarcoplasmic reticulum, adenyl cyclase, and 5-nucleotidase which are influenced by the levels of n-6 and n-3 fatty acids in the membrane lipids (Kinsella, 1990); and third, enzymes involved in the phosphoinositide cycle. This cycle, associated with the responses of many cells to a range of hormones, neurotransmitters and cell growth factors, gives rise upon activation of a specific phospholipase C to two important second messengers: inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol. IP_3 is responsible for the modulation of cytosolic calcium ions. Diacylglycerol, together with calcium ions and phosphatidyl serine, is involved in the activation of a protein kinase C which phosphorylates a number of intracellular proteins. Since poly-phosphoinositides are very rich in arachidonic acid in position 2, diacylglycerol arising from them is also rich in this fatty acid. A lipase acting on diacylglycerol may subsequently release arachidonic acid for conversion to eicosanoids.

The phosphoinositide cycle controls processes in cell division. The modulation of the cycle, therefore, influences the rate of division of cells in the immune response and in tumour growth. Dietary polyunsaturated fatty acids and the proportion of n-6 and n-3 fatty acids appear to affect the phosphoinositide cycle.

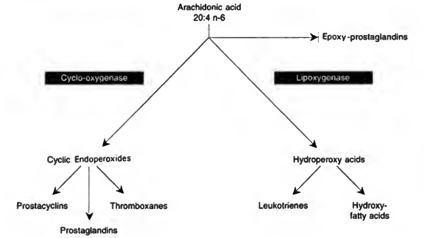
Eicosanoid formation. The n-6 and n-3 fatty acids in the membrane phospholipids exert metabolic control through their role as precursors of eicosanoids. These highly active compounds of 20 carbon atoms are released in very small quantities to act rapidly in the immediate environment. After enzymatic degradation, the products from eicosanoids in the urine are an indication of the production by the body.

The first step in the biosynthesis of eicosanoids is the release of a 20 carbon polyunsaturated fatty acid from phospholipids by phospholipases, mainly a phospholipase A_2, or from diacylglycerol produced in the inositol phosphate cycle. The eicosanoid cascade consists of hydroxylated derivatives of 20 carbon polyunsaturated fatty acids: (a) cyclic products, generated by a cyclo-oxygenase, which include prostaglandins, prostacyclin and thromboxane; (b) lipoxygenase products such as the 12-lipoxygenase derivatives, especially the products of the 5-lipoxygenase known as leukotrienes; and (c) products of cytochrome P_{450} activity (Figure 3.3). Eicosanoids, in general, are very potent, their effects are highly diverse, and the action of different eicosanoids often oppose each other. In addition, the patterns of eicosanoid production are different in various cells and tissues.

Among the most active eicosanoids, thromboxane A2(TxA2) derived from arachidonic acid is produced by platelets and other cells through the cyclo-oxygenase pathway. This eicosanoid is a platelet proaggregatory and smooth muscle contracting agent, and is rapidly inactivated to thromboxane B2. Prostacyclin ($PG1_2$), produced through the cyclo-oxygenase in cells of vessel walls, is a platelet anti-aggregatory and vasodilating agent. Other products of the cyclo-oxygenase pathway (for example, PGE_2 and $PGF_{2\alpha}$) exert various effects on smooth muscle cells, immune competent cells, and so forth. Among the products of the lipoxygenase pathway, the leukotrienes, produced mainly by the leukocytes, act on vascular parameters (permeability, contractility) and have chemotactic properties. They are involved in the modulation of inflammatory and immune processes.

Polyunsaturated fatty acids with 20 carbons and different degrees of unsaturation give rise to eicosanoids with different numbers and patterns of unsaturation, and with somewhat different biological activities. Since arachidonic acid (20:4 n-6) is the major polyunsaturated

FIGURE 3.3
The eicosanoid cascade



fatty acid in cells, the 2-series of eicosanoids is the most abundant and generally the most active. When a 20 carbon polyunsaturated fatty acid with a different number of double bonds, for example, di-homo-gamma linolenic (DGLA), 20:3 n-6, or eicosapentaenoic acid (EPA), 20:5 n-3, is incorporated into cell lipids, eicosanoids of the 1-series or the 3-series, respectively, are produced. These fatty acids also compete with arachidonic acid for the cycloxygenase, and thus reduce the formation of eicosanoids of the 2-series. EPA promotes the formation of eicosanoids of the 3 series and inhibits the formation of eicosanoids of the 2 series.

Effects on other lipid-derived mediators. Hydrolysis of selected cell phospholipids results in the formation of additional biologically active compounds such as the platelet activating agent, PAF. This compound arises from 1-alkyl, 2-acyl phosphatidyl choline. PAF is an extremely potent pro-inflammatory agent, and a potent activator of several types of cells in addition to platelets. Although data on the effects of various polyunsaturated fatty acids on the PAF pathway are limited, incorporation of arachidonic acid into cell phospholipids was shown to potentiate PAF production (Suga *et al.*, 1991). Different polyunsaturated fatty acids in position 2 of the precursor phospholipids can modify PAF formation, as was shown by the reduced production of PAF by monocytes of subjects receiving n-3 fatty acids (Sperling *et al.*, 1987). The opposing effects of n-6 and n-3 fatty acids may explain some influences of polyunsaturated fatty acids on the function of certain cells (*Ibid.*).

Effects on other parameters. The n-3 fatty acids appear to affect various other processes, such as the production of cytokines and other factors. Cytokines are a family of proteins produced and released by cells involved in inflammatory processes and in the regulation of the immune system. These include the interleukins and tumour necrosis factors. The mechanism by which n-3 fatty acids affect cytokine synthesis is not clear, but some studies have shown an effect on mRNA levels, suggesting a pretranslational level of action.

The effects of fatty acids on the expression of genes encoding for enzymes which are involved in lipid metabolism, as well as on the expression of genes involved in cell growth regulation (immediate early response genes), represent an additional important aspect of the biological roles of polyunsaturated fatty acids. It is apparent that fatty acids can interact with a group of nuclear receptor proteins that bind to certain regions of DNA and thereby, alter transcription of regulatory genes.

Much of the literature on the n-3 long-chain fatty acids deals with pharmacological doses or is concerned with lipoproteins and their relationship to coronary heart disease. Additional clinical and metabolic studies should be encouraged.

Oxidation of fatty acids

Those fatty acids that are not used for synthesis of eicosanoids or incorporated into tissues are oxidized for energy. Fatty acids yield energy by beta oxidation in the mitochondria of all cells, except those in the brain and kidney. They enter the mitochondria as specific acyl carnitine derivatives. Saturated short, medium, and long-chain fatty acids undergo the first step of beta-oxidation with different dehydrogenases. The process yields successive acetyl-CoA molecules which enter the tricarboxylic acid cycle or other metabolic pathways. Acetate is the eventual product from fatty acids with an even number of carbon atoms. Unsaturated fatty acids require two more enzymatic steps than saturated fatty acids to change *cis* double bonds to *trans* and to move them from the alpha to beta position. Even so, oxidation of unsaturated fatty acids, including linoleic acid is as fast or faster than that of palmitic acid. The initial oxidative reaction is carried out by a different enzyme from that in mitochondria; the fatty acyl CoA enters directly into this organelle. The process does not proceed to the complete production of acetate but a shortened fatty acid is transferred to the mitochondria for complete oxidation. Long chain (>20C) fatty acids are preferentially oxidized by peroxisomes; also fatty acids with less than 14C are oxidized by this system. Peroxisomal oxidation is energetically less efficient than mitochondrial oxidation and yields more heat. This type of oxidation can be induced by diets which are high in fat as well as by a variety of xenobiotics.

Chapter 4

Global trends in the availability of edible fats and oils

Consumers are often attracted to foods which have textures and flavours derived from fat. Although there are differences according to region, season and food habits, consumers generally increase the proportion of fat in their diet as their incomes rise. The increase in the quantity and change in the quality of fats and oils in the diet have important consequences for nutrition.

In discussing global trends in the availability of dietary fats, the figures in this chapter refer to the amounts of fats and oils which are available for human consumption. These figures are derived from FAO Food Balance Sheets which are prepared on the basis of statistics related to production, trade, stock and non-food use.

Current consumption of fat

In 1990, the amount of total dietary fat that was available worldwide was estimated to be 68 grams per caput per day. However, this average figure does not reveal the large disparities among geographic regions. While in Asia and Africa the amount of total fat available was less than 50 grams per caput per day, in South America it was 74 grams per caput per day. In the former USSR, the amount of total fat available was 107 grams per caput per day. In North and Central America, the total amount of fat available was 126 grams per caput per day while in Europe it was 143 grams per caput per day. Finally, 121 grams per caput per day were available in Oceania (FAO, 1993b).

When countries are categorized by level of economic development, the differences in the availability of total fat among groups appear more distinctly (Table 4.1). In developed countries, the daily per caput availability of fat was 128 grams while in developing countries it did not exceed 49 grams. In Africa (excluding Egypt, Libya and South Africa), the availability of fats and oils is low, while in North America, the level of fat available is high.

Within these two economic groups, there are large differences in total fat availability among regions and individual countries. Countries such as Rwanda, Cambodia and Bangladesh have less than 20 grams per caput per day while Ireland, Denmark, Luxembourg and Belgium have more than 170 grams per caput per day (*Ibid.*).

TABLE 4.1
Availability of fats and fat energy ratio by economic group (1961-1990)

Region	Fat g/caput/day		Increase between 61 and 90	Mean fat energy ratio	
	1961	1990	%	1961	1990
DEVELOPING	**28**	**50**	**78**	**13**	**18**
Africa	38	43	13	16	18
Far East	22	45	105	11	16
Near East	46	72	56	19	22
Latin America	51	75	47	20	25
DEVELOPED	**93**	**128**	**38**	**28**	**34**
former USSR	69	107	55	20	28
Oceania	125	138	10	36	36
Europe	104	143	37	32	37
North America	124	151	22	37	37

Source: FAO Food balance sheets, Agrostat PC, 1993

A description of the situation in different countries during the period 1988-90 is given in Table 4.2. Seventy-two countries, representing more than 63 percent of the total population for which data exist, had less than 60 grams of fat available for individuals on a daily basis.

TABLE 4.2
Total fats available per caput/day and mean fat energy ratio in 165 countries (1988-90)

Number of countries	Inhabitants number*1000:	Class of total fats per cap/day 88-90	Mean fat energy ratio
9	299,164	<30 g	10%
63	3,035,746	30-59.9 g	18%
64	1,197,754	60-119.9 g	27%
29	752,318	120 g and +	38%

Source: FAO Food balance sheets and population, Agrostat PC, 1993

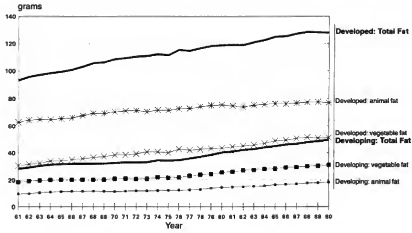
The Fat Energy Ratio (FER) is the proportion of dietary energy derived from total fat. Among individual countries, the average FER ranges from 7 to 46 percent. The means of FERs for countries in different regions of the world are shown in Tables 4.1 and 4.2.

Survey data on food consumption tend to confirm these trends and patterns of use of dietary fats and in some cases, demonstrate even more extreme situations. In Viet Nam, for instance, the average consumption of fats and oils was found to be very low (FAO, 1990). The FER calculated on the basis of consumption surveys was 6 percent while it was 11 percent according to the Food Balance Sheets. In some areas of Viet Nam (such as the "Highlands") data from these surveys indicate that the consumption of fat and the FER are even lower. On the other hand, the MONICA project in Germany indicated that the FER for men was more than 50 percent, if alcohol is excluded from the energy breakdown (Colling *et al.*, 1989).

Factors explaining consumption trends

FAO Food Balance Sheets show that the availability of fat for human consumption has increased steadily in both developed and developing countries (Figure 4.1). From 1961 to 1990, global availability of fat increased from 49 to 68 grams per caput per day. In developed countries, the amount of fat available grew from 93 to 128 grams while it increased from 28 to 49 grams in developing countries. Even though the increase in fat

FIGURE 4.1
Availability of fats per caput/day in developed and developing countries* (1961-1990)



Source: FAO Agrostat PC, 1993
* 165 countries

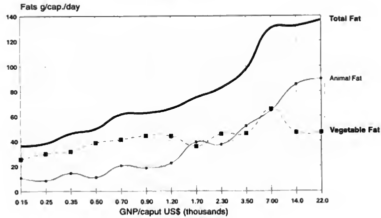
availability was twice as high in developing countries as in developed ones, there was still a large difference in the availability of fat according to levels of economic development.

Within developing regions, the rates of increase have varied (Table 4.1). In 1961, the fat availability in the Far East was lower than in Africa. Since then, the availability of fat has increased sharply and it is now higher than in Africa. The availability of fat was high in Oceania in 1961 and the increase has been minor. Fat availability was low in Africa 30 years ago and the increase in availability has been slow.

Income. The first factor which explains these changes is income, the basic indicator of economic development. The availability of animal and vegetable fats is closely linked to income (Périssé, Sizaret and François, 1969). The influence of the Gross National Product per caput on food availability in 134 countries is illustrated in Figure 4.2.

There was a steady increase in fat availability in countries with incomes ranging from US$150 to US$350 per year. For those countries with yearly per caput incomes between US$350 and US$7 000, there was a sharp increase in the amount of total fat available, while the availability of fat remained at the same level for countries with individual incomes above US$7 000. In countries with per caput incomes under US$ 7 000, consumption of both vegetable and animal fats increased at similar rates. Beyond US$ 900, the availability of animal fats increased rapidly. The availability of vegetable fats actually declined when per caput incomes exceeded US$7 000. Food consumption surveys confirm this trend especially in countries such as Bangladesh (Hassan and Ahmad, 1992) or Brazil [Instituto Brasileiro de Geografia e Estadistica (IBGE), 1978] where GNP/caput is low. In these countries, poverty is the main factor limiting fat consumption, especially that derived from animals.

FIGURE 4.2
Fat available by income per person in 134 countries (1989)

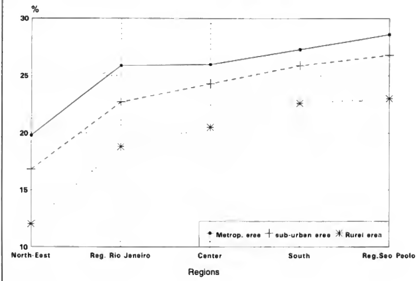


Source: FAO Agrostat Mainframe, 1993

Urban life-styles. Urbanization is strongly associated with the increasing consumption of fat in developing countries. This a general phenomenon that is part of an overall change of food habits. In Bangladesh, for instance, the consumption of fat in urban areas is eight times higher than in rural areas (Hassan and Ahmad, 1992). The same is true in Niger, where consumption in urban areas is two or three times higher than in rural ones, depending on the season [Comité Inter-état de Lutte contre la Sécheresse dans le Sahel (CILSS), 1991]. In Niger, the FER is 7 percent among people living in rural areas, while it is 14 percent among those who have recently come from rural areas to urban areas. It is 19 percent among individuals permanently living in Niamey, Niger's capital (*Ibid.*). A survey carried out in Brazil in 1974-75 (IBGE, 1978) indicated a clear association between urbanization and fat consumption independent of climatic regions (Figure 4.3). The increased FER which is commonly seen as areas become cities is striking since energy intakes tend to decrease with urbanization, due to lower levels of physical activity.

Other factors. The physical environment, local availability of fats and oils, food habits and the level of education are other factors which affect the level of fat consumption. Sociological and individual factors also affect fat consumption. Food Balance Sheets indicate that during the last decade, a decrease in the availability of fats of animal origin, particularly in many developed countries in North Europe, North America and Oceania began. Food consumption surveys confirm this evolution (Stephen and Wald, 1990; Den Hartog, 1992).

FIGURE 4.3
Fat energy ratio in rural, suburban and metropolitan areas of 5 Brazilian regions



Source: IBGE, 1978

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Analyses of fat consumption data show that in the most developed countries individuals belonging to the lowest socio-economic groups consume more fatty foods (Nova Scotia Department of Health, 1993). Studies show that men consume more fatty foods than women and young people eat more fatty foods than the elderly (Hulshof *et al.*, 1991; Read *et al.*, 1989; Popkin, Haines and Patterson, 1992; Nova Scotia Department of Health, 1993).

All of these factors affect the total quantity of fat consumed. Furthermore, in developed countries food choices are increasingly linked to the type of fat contained in food.

Shifts in fat consumption

According to FAO Food Balance Sheets (FAO, 1993b), visible fats provide nearly half of the available fat in the world (Table 4.3). Furthermore, oils of vegetable origin contribute a markedly higher percentage of visible fat than those of animal origin. The percentage of visible fats of animal origin is higher in developed countries, except North America (Canada and USA). From data describing 165 countries, the availability of animal fats has decreased or remained at the same level in 102 countries. In the United Arab Emirates, Norway, Finland, the United Kingdom, Ireland, Canada, Australia, Denmark, the United States and the Netherlands the consumption of animal fats has been reduced. This trend became sharper at end of the 1980s. In contrast, the sharpest increase (8 to 17 grams per caput per day) has been recorded in the eastern European countries, Cuba, Belgium, Luxembourg, Italy, France and Cape Verde.

Worldwide, vegetable sources supplied 24 grams of oil per caput per day in 1990, while animals provided 6 grams of visible fat per caput per day. Out of the 165 countries, all but 12 countries have experienced increases in their vegetable oil availability since 1961 (FAO,

TABLE 4.3
Contribution of fats and oils by foods groups in 1990

REGION	Total Fat gram /cap/day	% of Total Fats						
		Visible Fats		Invisible Fats				
		Vegetable	Animal	Meat	Milk	Cereal	Oilcrop	Others
World	68.3	36	11	23	9	8	5	8
Africa	43.1	48	4	9	5	16	10	8
Far East	44.6	35	6	24	6	13	9	7
Near East	72.3	49	7	11	7	13	4	9
Latin America	75.4	43	9	22	10	7	3	6
USSR	106.8	25	22	26	12	5	1	9
Oceania	137.8	20	18	40	13	2	2	5
Europe	142.8	30	20	28	12	3	1	6
North America	151.0	39	9	27	14	2	3	6

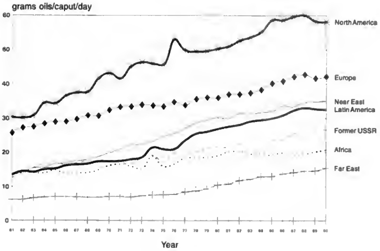
Source: FAO Food balance sheets, Agrostat PC, 1993

1993). In 1990, 65 countries had more than 30 grams per caput per day of vegetable oil and 6 other countries (Malaysia, Tunisia, Spain, Italy, Israel and Greece) had more than 60 grams per caput per day. The change in vegetable oil availability in different regions of the world is shown in Figure 4.4.

While each country has particular varieties of oil, only certain oils are important on a global level. Among the major commercial oils, the supplies of soya bean oil have increased from 2.2 to 7.0 grams per caput per day, while the availability of sunflower oil has grown from 1.3 to 3.5 grams per caput per day. Supplies of rapeseed oil increased from 0.9 to 3.4 grams per caput per day and the availability of palm oil rose from 0.9 to 2.9 grams per caput per day. Peanut oil supplies remained almost the same (2 grams per caput per day) during the last 3 decades (FAO, 1993).

Of particular interest is the content of essential fatty acids. Soybean oil contains less linoleic acid than sunflower seed oil. However, both oils provide adequate amounts of linoleic acid. On the other hand, essential fatty acids from the n-3 family may be supplied from vegetable oils containing α-linolenic acid, such as canola and soybean oils, and from fish oils containing the longer-chain fatty acids. Fish oils do not appear as separate items in food balance sheets because they are consumed as part of the fish in many countries. Only a few countries show that significant amounts of fish oils are available. Seven countries have more than 2 grams per caput per day.

FIGURE 4.4
Change in vegetable oil availability in developed and developing regions



Source: FAO Agrostat PC, 1993

Conclusion

During the last three decades, the availability of fat has increased steadily throughout the world, especially in developing countries. Nevertheless, fat consumption remains low in these countries in comparison with developed nations.

In developing countries, rural families, who are often the poorest members of society, consume diets with a low fat content because of their low income and limited access to a diversified food supply. Malnutrition is a crucial problem and increasing the amount of energy available should be a priority. Fats and oils play a crucial role in bringing about this increase. Nevertheless, in developing countries policies designed to promote this increase can be a risk factor for urban populations who may consume diets with an excess of fat.

In some developed countries, especially in Europe, the availability of polyunsaturated fatty acids is low in comparison to saturated fatty acids because the amount of animal fat available is more than twice that of vegetable fat. In 12 countries, the availability of animal fat is more than 30 grams per caput per day.

The most developed countries have reached very high levels of fat consumption. Some of these countries show a tendency to decrease fat consumption, this is mainly a decrease in visible fats of animal origin which are rich in saturated fatty acids. This new trend is linked to the policies implemented by the most developed countries (especially in North America and Northern Europe) which aim to improve the current consumption patterns, especially by reducing intakes of saturated fatty acids and increasing intakes of polyunsaturated fatty acids. In these countries, visible fats provide more than 70 grams per caput per day. Policies aimed at improving the quality of fats and oils can be effective with the collaboration of food industries.

Chapter 5
Processing and refining edible oils

Processing can remove the components of edible oils which may have negative effects on taste, stability, appearance or nutritional value. To the extent possible, processing should preserve tocopherols and prevent chemical changes in the triacyglycerols.

Rural vegetable oil production

Rural oil extraction usually occurs near the areas of raw material production. This provides the small-scale processor with access to raw materials, helps to ensure that perishable oil crops are processed quickly, and reduces transport costs. For rural communities and the urban poor, unrefined vegetable oils contribute significantly to the total amount of oil consumed. Crude oils are affordable to low-income groups and serve as important sources of ß-carotene and tocopherols.

To maintain the quality of the raw material, care is needed during and after the harvesting of oil-bearing fruits that are perishable and susceptible to fat breakdown. Bruising of fresh palm fruits accelerates lipase activity leading to fat degradation. Oil-bearing crops such as sheanuts are prone to mould infestation during storage. This is curtailed by heat treatment: steaming or boiling, coupled with sun-drying to reduce the moisture content.

Storage. The moisture content of oil seeds and nuts influences the quality of raw materials over time. In most rural operations, sun-drying reduces the moisture content of oil seeds to below 10 percent. Adequate ventilation or aeration of the seeds or nuts during storage ensures that low moisture levels are maintained and microbial development is avoided. This is important in the storage of groundnuts which are highly susceptible to aflatoxin contamination through the growth of *Aspergillus flavus*. Since aflatoxins and pesticides are not removed by rural extraction techniques, microbial contamination and the application of insecticides should be avoided. There is a need for storage practices which are affordable and available to the small-scale processor. Perishable raw materials such as palm fruits should be processed as soon as possible after harvesting.

In humid developing countries, the sun-drying of oil seeds with a high moisture content, such as mature coconut, is slow and inefficient. Such conditions promote mould growth which results in high free fatty acid levels and poor organoleptic qualities. Coconut oil for human consumption should be obtained soon after harvest.

Pre-treatment. The first operation after harvesting involves sterilization and heat treatment by steaming or boiling, this inactivates lipolytic enzymes which could cause rapid degradation of the oil and facilitates the pulping of the mesocarp for oil extraction. "Sterilised" palm fruits are pulped in a wooden pestle and mortar or mechanised digestor.

Decortication or shelling separates the oil-bearing portion of the raw material and eliminates the parts that have little or no nutritional value. Small-scale mechanical shellers are available for kernels and nuts although manual cracking is still prevalent.

Most oil seeds and nuts are heat-treated by roasting to liquify the oil in the plant cells and facilitate its release during extraction. All oil seeds and nuts undergo this treatment except palm fruits for which "sterilization" replaces this operation.

To increase the surface area and maximize oil yield, the oil-bearing part of groundnuts, sunflower, sesame, coconut, palm kernel and sheanuts is reduced in size. Mechanical disc-attrition mills are commonly used in rural operations.

Extraction. In oil extraction, milled seed is mixed with hot water and boiled to allow the oil to float and be skimmed off. The milled oil seed is mixed with hot water to make a paste for kneading by hand or machine until the oil separates as an emulsion. In groundnut oil extraction, salt is usually added to coagulate the protein and enhance oil separation.

A large rotating pestle in a fixed mortar system can be powered by motor, humans or animals to apply friction and pressure to the oil seeds to release oil at the base of the mortar. Other traditional systems used in rural oil extraction include the use of heavy stones, wedges, levers and twisted ropes. For pressing, a plate or piston is manually forced into a perforated cylinder containing the milled or pulped oil mass by means of a worm. The oil is collected below the perforated chamber. A variety of mechanical expellers have been designed. The pre-heated raw material is fed into a horizontal cylinder by a wormshaft. By means of an adjustable choke, internal pressure which is built up in the cylinder ruptures the oil cells to release the oil.

Dehydration. By boiling in shallow pans, traces of water in crude oil are removed after settling. This is common in all rural techniques which recognize the catalytic role of water in the development of rancidity and poor organoleptic qualities.

Pressed cakes. A by-product of processing, the pressed cake, may be useful depending on the oil extraction technique applied. Cakes from water-extracted oil are usually depleted of nutrients. Other traditional techniques, for instance, those used for groundnut and copra ensure that the by-products, if handled with care, are suitable for human consumption.

Traditional technologies. In many countries, traditional processes for producing oil are very important, especially among communities which have easy access to raw oleaginous materials. Traditional processing tends to be environmentally sound and the skills required are family or group activities, involving women in particular. In a changing industrial atmosphere, these positive features have been outweighed by the negative aspects of traditional processing such as small production capacities, poor economies of scale, high expenditures of energy and time, and the cost of transporting oils to markets.

Large-scale production

Storage. Many steps in industrial processing find their origin in the traditional processes. In large-scale operations, oilseeds are dried to less than 10 percent moisture. They may be stored for prolonged time periods under suitable conditions of aeration with precautions against insect and rodent infestation. Such storage reduces mould infection and mycotoxin contamination and minimizes biological degradative processes which lead to the development of free fatty acids and colour in the oil.

Oil-bearing fruits such as olive and palm are treated as quickly as possible. Palm is sterilized as a first step in processing. Adipose tissues and fish-based raw materials (that is, the body or liver) are rendered within a few hours by boiling to destroy enzymes and prevent oil deterioration.

Processing. Oilseeds are generally cleaned of foreign matter before dehulling. The kernels are ground to reduce size and cooked with steam, and the oil is extracted in a screw or hydraulic press. The pressed cake is flaked for later extraction of residual fat with solvents such as "food grade" hexane. Oil can be directly extracted with solvent from products which are low in oil content, that is, soybean, ricebran and corn germ.

After sterilization, oil-bearing fruits are pulped (digested) before mechanical pressing often in a screw press. Palm kernels are removed from pressed cakes and further processed for oil. Animal tissues are reduced in size before rendering by wet or dry processes. After autoclaving, tissues of fish are pressed and the oil/water suspension is passed through centrifuges to separate the oil.

Oil Refining. Refining produces an edible oil with characteristics that consumers desire such as bland flavour and odour, clear appearance, light colour, stability to oxidation and suitability for frying. Two main refining routes are alkaline refining and physical refining (steam stripping, distillative neutralisation) which are used for removing the free fatty acids.

The classical alkaline refining method usually comprises the following steps:

Step 1. Degumming with water to remove the easily hydratable phospholipids and metals.

Step 2. Addition of a small amount of phosphoric or citric acid to convert the remaining non-hydratable phospholipids (Ca, Mg salts) into hydratable phospholipids.

Step 3. Neutralising of the free fatty acids with a slight excess of sodium hydroxide solution, followed by the washing out of soaps and hydrated phospholipids.

Step 4. Bleaching with natural or acid-activated clay minerals to adsorb colouring components and to decompose hydroperoxides.

Step 5. Deodorising to remove volatile components, mainly aldehydes and ketones, with low threshold values for detection by taste or smell. Deodorisation is essentially a steam distillation process carried out at low pressures (2-6 mbar) and elevated temperatures (180-220°C).

For some oils, such as sunflower oil or rice bran oil, a clear table product is obtained by a dewaxing step or crystallization of the wax esters at low temperature, followed by filtration or centrifugation.

The alkaline neutralisation process has major drawbacks, the yield is relatively low and oil losses occur due to emulsification and saponification of neutral oil. Also, a considerable amount of liquid effluent is generated. The soaps are generally split with sulphuric acid to recover free fatty acids along with sodium sulphate and some fat-containing acid water steam.

In physical refining, the fatty acids are removed by a steam distillation (stripping) process similar to deodorisation. The low volatility of fatty acids (depending upon chain length) requires higher temperatures in physical refining than those required for only deodorisation. In practice, a maximum temperature of 240-250°C is sufficient to reduce the free fatty acid content to levels of about 0.05-0.1 percent. A prerequisite for physical refining is that phosphatides be removed to a level below 5 mg phosphorus/kg oil. In the classic refining process, this level is easily achieved during the neutralisation stage, but special degumming processes may be required for physical refining of high-phosphatide seed oils. These procedures rely on improved hydration of phospholipids by intimate contact of the oil with an aqueous solution of citric acid, phosphoric acid and/or sodium hydroxide, followed by bleaching (Segers and van de Sande, 1988).

It is unlikely that the mild reaction conditions during degumming and neutralisation will induce any significant undesirable changes in the oil composition. On the contrary, several impurities including oxidised components, trace metals and colouring materials are partially removed by entrainment with the phospholipids and soapstock. These impurities are further reduced during bleaching. Neutralisation also contributes significantly to the removal of contaminants such as aflatoxin and organophosphorous pesticides (Thomas, 1982). Organochlorine pesticides and polycyclic aromatic hydrocarbons, if present, must be removed during the deodorisation/stripping stage and by active carbon treatment. Some loss of tocopherols and sterols during alkaline neutralisation usually occurs, however, under well-controlled conditions (minimising air contact) this need not exceed 5-10 percent (Gertz, 1988; Johansson and Hoffmann, 1979).

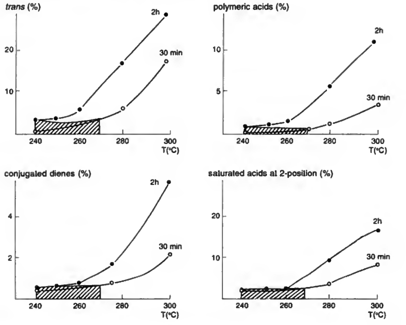
Potential side reactions during high-temperature processing

The possibility of negative effects of high temperatures during deodorisation and stripping has evoked concern. In several studies, extreme conditions of temperature and time (even with free access of air) were applied to produce significant quantitative results. However, the results of model studies should be related to practical processing conditions. As early as 1967-1979, the German Society for Fat Research (DGF) defined upper limits for deodorisation conditions [240°C for 2hrs, 270°C for 30 min.] (Fremdstoff-Kommission, 1973; Gemeinschaftsarbeiten, 1992).

Good manufacturing practice also implies the following: the use of stainless steel equipment; the careful deaeration at < 100°C before heating to the final stripping temperature; the use of oxygen-free steam; and strict feedstock specifications (typically: Fe 0.1, Cu 0.01, P 5, bleaching earth 5 mg/kg oil max.).

Investigations in which oil was maltreated under extreme conditions (Rossel, Kochhar and Jawad, 1981; Jawad, Kochhar and Hudson, 1983 a, b; 1984) determined the effects of temperature (240-300°C) and time (30-180 min.) during "physical refining" of soybean oil (degummed with phosphoric acid and lightly bleached, but still containing 20 mg P, 0.35 mg Fe and 0.05 mg Cu per kg oil). A strong effect of temperature on the formation of *trans* fatty acids and polymeric compounds is shown in Figure 5.1. Time also has a significant effect. At 280-300°C, there was evidence for appreciable inter- or intra-esterification (increase in the content of saturated fatty acids at the 2-position of the triacylglycerols);

FIGURE 5.1
Formation of various artefacts in soybean oil under extreme conditions of heat treatment

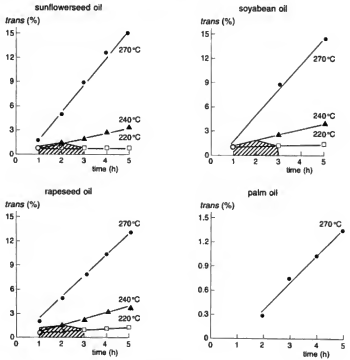


Note: Shaded areas indicate upper limits of conditions as applied during physical refining.
Source: Jawad, Kochhar and Hudson, 1983.

substantial amounts of conjugated fatty acids were formed as well. The shaded areas indicate the usual range of processing conditions required for physical refining (270°C for 30 min; 250°C for 1 h; 240°C for 2 h; 220°C for 3 h). Under these conditions, all changes induced by the high temperature treatment appear to be relatively minor.

***Cis-trans* isomerisation.** One of the most sensitive parameters used to detect chemical changes resulting from severe processing conditions is *cis-trans* isomerisation, especially of linolenic acid. The most complete study in this area was made by Eder (1982), who investigated the formation of geometric isomers in various oils on the laboratory, pilot plant and production scales. On a laboratory scale, with unbleached soyabean oil at 240°C, the formation of C18:3 isomers (determined by GLC) was insignificant (less than 1 percent, even after 5 h, against 3 percent at 260°C). The total *trans* contents (determined by infrared spectroscopy) for several types of oils deodorised in stainless steel equipment on the pilot plant scale is depicted in Figure 5.2. The data show a strong effect of temperature, especially

FIGURE 5.2
Trans fatty acid formation during high temperature deodorisation of various oils



Note: Shaded areas indicate upper limits of conditions as applied during physical refining.
Source: Eder, 1982

between 240 and 270°C, and confirm the general trend. That is, even for highly unsaturated oils containing linolenic acid, the formation of *trans* isomers is slow under the conditions recommended for industrial deodorisation/physical refining (for example, 250°C max.). In conclusion, in the temperature range of 240-250°C, the amount of *trans* fatty acids formed from unsaturated oils is about 1 percent or less per hour. This figure is consistent with the data discussed earlier (Rossell, Kochhar and Jawad, 1981; Jawad, Kochhar and Hudson, 1983b). Heating of oils in air, under simulated frying conditions, has led to similar types of isomerised fatty acids (Grandgirard, Sebedio and Fleury, 1984; Grandgirard and Juillard, 1987; Sebedio, Grandgirard and Provost, 1988).

The formation of positional isomers (that is, double bonds shifted along the fatty acid chain) of linolenic and linoleic acid under deodorisation/physical refining conditions has not been reported.

Dimerisation and polymerisation. Quantitative data on the formation of polymeric compounds from bleached soybean oil are included in Figure 5.1 (Jawad, Kochhar and Hudson, 1983b). Up to about 260°C, their rate of formation appears to be low; above 260°C, the increase is more rapid. Similar trends were observed by Eder (1982). A rapid increase of the amount of polymeric triacylglycerols was only observed at 270°C. Deodorisation of soybean oil on a commercial scale (2 x 51 min at 240°C) resulted in an increase of 0.5 percent to 0.8 percent polymeric triacylglycerols.

When the temperature was increased to 270°C in one of the deodoriser trays, 1.5 percent polymers was found. This suggests that the content of di- and polymeric triacylglycerols do not normally exceed 1 percent by weight in properly refined oils and fats.

Strauss, Piater and Sterner (1982) carried out toxicological studies on mice fed concentrates (24 or 96 percent) of dimeric (incl. polymeric) triacylglycerols isolated from soybean oil that had been deodorised for 3.5 h at 220°C and 1 h at 270°C and contained 1.5 wt percent of dimers. The acute toxicity was found to be low with an LD 50 above 18 g/kg body weight. Long term administration of the dimer concentrate (12 months, 15 wt percent of the 24 percent concentrate in the diet) did not reveal any significant differences compared with the control group. The absorption of dimeric fatty acids was found to be poor. Therefore, the presence of small amounts of dimers and polymers in processed oils does not seem to present a physiological problem.

Physical losses

During deodorisation or physical refining, volatile components are removed from the oil by the combination of high temperature, low pressure and stripping action of inert gas (steam). The degree of removal depends on the physical properties of the components (especially vapour pressure) and on the temperature and volume of steam passed through the oil. Some physical losses are highly desirable, for example, the removal of off- flavours, pesticides and polycyclic aromatic hydrocarbons, if present. Other losses of nutritionally valuable components, such as tocopherols and sterols, are potentially undesirable.

Quantitative data on the composition of deodoriser distillates and the removal of various types of contaminants from oils during deodorisation were reported (Chaudry, Nelson and Perkins, 1978; Larsson, Eriksson and Cervenka, 1987; Sagredos, Sintra-Roy and Thomas, 1988; Gemeinschaftarbeiten, 1990). During high temperature deodorisation or physical refining, especially, the concentrations of organochlorine pesticides (lindane, DDT etc.), if present in the bleaching oil, are reduced to very low levels.

Polycyclic aromatic hydrocarbons (PAH) have been a reason for concern since they were detected in some types of crude oils and fats in the 1960s. An example of this is coconut oil from copra dried with non-purified smoke gases. Depending upon the number of aromatic rings, polycyclic aromatic hydrocarbons are classified as either light (3-4 rings) or heavy (5 or more rings). Some of these compounds have proven carcinogenic properties, for example, benz-∝-pyrene. Light polycyclic aromatic hydrocarbons can be removed by deodorisation or physical refining, whereas heavy polycyclic aromatic hydrocarbons can be removed only by adsorption onto activated carbon (Chaudry, Nelson and Perkins, 1978; Larsson, Eriksson and Cervenka, 1987). This treatment, which can be combined with the bleaching process, is effective in reducing the amount of polycyclic aromatic hydrocarbons to acceptable levels.

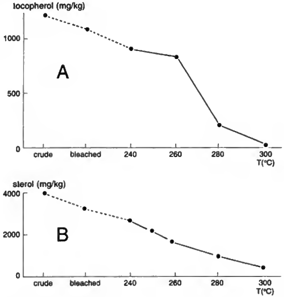
Some loss of tocopherols and sterols by evaporation during high temperature deodorisation and physical refining is inevitable. However, they have higher molecular masses and lower volatilities than free fatty acids and light polycyclic aromatic hydrocarbons. Consequently, the losses of tocopherols and sterols need not be severe if the processing conditions are well-chosen. Extreme conditions applied in some model studies do induce more drastic effects. Typical results obtained for soybean oil (Rossell, Kochhar and Jawad, 1981; Jawad, Kochhar and Hudson, 1984) are illustrated in Figure 5.3. After 2 hrs at 300°C (a drastic treatment), the tocopherols and sterols almost completely disappeared, whereas the actual reduction during physical refining at 240°C for 120 min is only about 15-20 percent. The total refining losses (including pretreatment) are about 25-35 percent. Many investigators report similar data for a variety of oils. The losses tend to be higher in physical refining than in alkaline refining because of higher stripping temperatures.

Under extreme conditions, some isomerisation of ϐ-sitosterol can occur and individual tocopherols (α, ϐ, τ, δ) and sterols may behave differently when exposed to high temperatures (Jawad, Kochhar and Hudson, 1984). However, these phenomena are of minor significance under more realistic conditions. The percentage composition of the tocopherol and sterol fractions remains essentially unchanged during processing (Gemeinschaftarbeiten, 1990; Jung, Mood and Min, 1989).

For refined oils such as sunflowerseed, cottonseed and rapeseed oil, an upper limit of 30-35 percent loss of tocopherols throughout the entire processing would still satisfy the generally accepted criterion of ∝-tocopherol equivalents/linoleic acid $\geq$ 0.6 mg/g (Jager, 1975). Soybean oil, which is high in γ-tocopherol and consequently well-protected *in vitro*, is relatively low in α-tocopherol and cannot reach this ratio.

The β-carotene in palm oil is another valuable component that needs consideration in the refining process. Special processes for retention are being designed. Olive oil and sesame oil are used in unrefined form since a specific taste is expected by consumers.

FIGURE 5.3
Reduction of tocopherol (A) and sterol (B) content of soybean oil during bleaching and severe heat treatment (time = 2 hours)



Source: Rossell, Kochhar and Jawad, 1981.

Fat modification processes

Hydrogenation. Hydrogenation of edible oils and fats has been applied on a large scale since the beginning of this century. The process is carried out in a three-phase system (hydrogen gas, liquid oil and solid catalyst) at temperatures ranging from about 120°C to about 220°C max. in the final stages of the reaction. The catalyst consists of small nickel crystallites supported by an inorganic oxide, usually silica or alumina. After the reaction, the catalyst is filtered off and any traces of residual nickel are removed in post-refining to a level of about 0.1 mg/kg or below.

Hydrogenation is a series of consecutive reactions following pseudo first order reaction kinetics:

$$18{:}3 \xrightarrow{K_3} 18{:}2 \xrightarrow{K_2} 18{:}1 \xrightarrow{K_1} 18{:}0$$

in which K_3, K_2 and K_1 are the reaction rate constants for linolenic, linoleic and oleic acid, respectively. The K_3 is much larger than K_2 and K_1. In nearly all hydrogenations, linolenic acid is transformed into compounds which are less unsaturated. Depending upon the reaction conditions, the so-called Selectivity Ratio (K_2/K_1) can change significantly; for example, for nickel catalysts it varies from approximately 10 at a low temperature to 50 or 100 at high temperature. A high Selectivity Ratio implies that relatively little saturated acid is formed and that monounsaturated fatty acids are the dominant reaction product.

Apart from reduction of unsaturation, isomerisation of double bonds also takes place during hydrogenation: geometric isomerisation (*cis-trans*) and positional isomerisation. The mechanisms of hydrogenation and isomerisation are strongly interrelated (Koritala and Dutton, 1973; Rozendaal, 1976). Initially, a half-hydrogenated intermediate is formed in which the molecule adsorbed to the catalyst surface by a single bond can freely rotate. Addition of a second hydrogen atom results in saturation, whereas abstraction of a hydrogen atom from the half-hydrogenated intermediate to the nickel surface yields either the original molecule or a positional or geometric isomer. Hydrogenation of polyenoic fatty acids occurs, at least partially, through conjugated isomers (for example, c9, t11 or t10, c12) which are highly reactive and, therefore, rapidly converted into *cis* or *trans* monoenoic acids without accumulating. Of the total amount of *trans* fatty acids present in hydrogenated oils and fats, the greater part by far are *trans* monoenes. Due to the important role hydrogenation plays in the production of plastic fats, *trans* fatty acids may be found in considerable amounts in many products. The amount of *cis,trans* and *trans,cis* dienes is much smaller, whilst the level of *trans,trans* dienes seldom exceeds 1 percent. (Gottenbos, 1983).

Apart from hydrogenation, there are two other important fat modification technologies. The first is interesterification, the random rearrangement of the fatty acids in the triglyceride molecule under the influence of a moderately alkaline catalyst. This modifies the melting behaviour of the fat without changing the nature of the fatty acids. The second is fractionation, the controlled separation of oil/fat fractions by low temperature (dry fractionation), or solvents (solvent fractionation). In this process, there are no changes in the chemical nature of the fatty acids. Palm oil is fractionated into palmolein and palmstearin.

Interrelationships of fat modification technologies. Industry uses various oils and fats interchangeably while retaining constant quality. Generally, the least expensive combination of raw materials compatible with the required quality is chosen. Hydrogenation greatly extends the number of fats available with specified melting behaviour and this increases interchangeability and lowers costs. In non-hydrogenation situations, a combination of interesterification, fractionation and selection of the starting oil may be acceptable solutions for limiting the formation of isomers in terms of product quality, however, the costs are higher. While the actual specific modification, for example, hydrogenation or interesterification, is relatively inexpensive, the costs of losing flexibility can be significantly higher. The processes for oil modification may change as new compositions become available through plant biotechnology (Sommerville, 1993).

Other considerations

Storage, transport and packaging of oils. Oils and fats must be protected against oxidative deterioration, contamination with water, dirt, or other fats, absorption of foreign odours and tastes, thermal deterioration, and entry of foreign substances from packaging and lining materials. Temperature, oxygen pressure, oxidation products, trace metals, oxidative and lipolytic enzymes, reduction in natural antioxidants and visible and ultraviolet light are all factors in such deterioration. The use of low storage temperatures, nitrogen or vacuum packaging; the avoidance of copper, copper alloys and iron as construction material of storage vessels; and the use of synthetic or natural antioxidants and metal sequestrants as additives, work to prevent deterioration of oil during storage.

Selection of oil processing technology. The use of oil processing technology and its application in products is influenced by several factors. For example, the demand for triacylglycerols with specific fatty acids in the 1, 2 and 3 positions of the molecule can be met through enzymatic interesterification which uses lipases as catalysts in the interesterification process. The more readily absorbed fatty acid in the 2-position can provide for specific triacylglycerols with medical benefits. For example, essential fatty acids can be supplied to patients with various types of fat malabsorption or energy can be given to infants with palmitic acid in the 2-position. Another is the increased use of physical refining because of consumer pressure for less "chemical" processing.

Conclusions

Rural refining of oil-bearing plants can produce fats and oils of good quality which provide needed energy and fat soluble vitamins. Commercial refining produces fats and oils that can be of high quality and have the characteristics of bland taste, clear colour, good keeping quality and frying stability. Commercially-refined fats and oils are free from known contaminants extracted from the raw agricultural products. Refining may remove nutritionally valuable carotenoids to yield oils of low colour, but retains substantial proportions of tocols, and does not change fatty acid nor triacylglycerol compositions. Temperature, time and pressure must be carefully controlled during industrial refining. Oil products should be properly stored, transported and packaged to maintain quality and consumers must assume responsibility for not abusing oils and fats within households.

Industry can design almost any fat or oil for a specific application by the use of various modification processes, such as hydrogenation, interesterification, fractionation or blending. Hydrogenation typically reduces essential fatty acid content and creates various fatty acid isomers, both *cis* and *trans*. The wide flexibility available to industry through the selection of raw materials and different modification processes allows for the production of oils at the lowest cost possible, an important aspect of food production.

Chapter 6

Selected uses of fats and oils in food

Fats are the primary constituents of margarines, butterfat, shortenings, and oils for salad and cooking. In addition to the visible fat contained in food, fats and oils are found in high quantities in many bakery goods, infant formulas, and dairy products and some sweets. Oils, butter or margarine are sometimes used directly on food.

Cooking oils

The major use of cooking oil is in frying, where it functions as a heat transfer medium and contributes flavour and texture to foods. One requirement of a cooking oil is that it be stable under the very abusive conditions of deep-fat frying, namely, high temperatures and moisture. In general, oil should be kept at a maximum temperature of 180°C during frying. Frying food at a temperature which is too low results in increased fat uptake. Water, which is contributed by the foods that are fried in an oil enhances the breakdown of fatty acids which occurs during heating. Hydrolysis results in a poor-quality oil that has a reduced smokepoint, darkened colour and altered flavour. During heating, oils also polymerize, creating a viscous oil that is readily absorbed by foods and that produces a greasy product. The more saturated (solid) the oil, the more stable it is to oxidative and hydrolytic breakdown, and the less likely it is to polymerize.

Oils rich in linolenic acid, such as soybean and canola oils, are particularly susceptible to these undesirable changes. When soybean oil is partially hydrogenated to reduce the linolenic acid from about 8 percent to below 3 percent, it is a relatively stable frying oil and it is used in processed fried foods, pan and griddle frying, and sauces. Stability is increased by using cottonseed, corn oil, palm oil or palmolein or by more hydrogenation of soybean oil.

Foods that are fried and stored before eating, for example, snack products, require an even more stable oil. More saturated oils improve stability, however, if the frying fat is solid at room temperature it will produce a dry dull surface that is undesirable on some fried products. When oils are used continuously, as in restaurants, a frying fat which can withstand very heavy use is needed. In these cases, more solid shortenings are used to maximize the stability of the fat for many hours of frying.

Frying oils made from sunflower and safflower have lower stability because of their high polyunsaturated fatty acids and low γ-tocopherol content; however, high-oleic safflower and sunflower oils that have been genetically developed are suitable frying oils.

For optimal use of cooking oils, it is necessary to distinguish between different frying conditions. The most important parameters to be monitored are duration of use and nature of the foods to be fried. If food fats enter the frying oil, food components could destabilize the oil and the water content of the material could influence the frying operation. Whether the use is continuous or intermittent is relevant, continuous use provides a protective water vapour blanket that protects against oxidation. Finally, temperature must be considered.

Industrial use of fats and oils is usually well-monitored. The continuous operation (implying the constant addition of fresh oil) and quality requirements for the products normally ensure good quality control of the oil. In homes, where oils are normally used for much shorter periods of time and are discarded after being used once or twice, stability problems play a lesser role. Stability of frying oils is a more important factor in catering operations, where heating is intermittent and oils may be used for long periods.

Margarines

Margarines must have some crystalline structure to maintain a semisolid consistency at refrigerator and room temperatures. Sharp melting at body temperature is needed so that the margarine will melt rapidly in the mouth leaving no waxy feeling.

Oleic acid melts at 16°C, whereas elaidic acid melts at 44°C, so that the presence of some *trans* isomers may vastly raise the melting point and stability of a product. Stick-type margarines contain 10-29 percent *trans* fatty acids while tub-type margarines have 10-21 percent *trans* fatty acids. In addition to partial hydrogenation, the correct consistency of a margarine can be obtained by blending soft and hard fats. Lower fat spreads, for example, 40 percent or 60 percent fat, contain less *trans* fatty acids.

Another important feature in solidifying oil for margarines is the type of crystal formed. Fats are polymorphic, that is, they are capable of forming several different types of crystals. The α crystals are the tiniest, forming a smooth but unstable crystal. The β' crystals are medium in size, still they are generally desirable in margarines because they impart a smooth texture, are fairly stable, and ensure plasticity of the product. The largest crystals are the β type which are stable and grainy, and generally undesirable. Also, the β form is readily converted to a hard and brittle structure. Products such as liquid shortenings and coating fats sometimes require the β crystal.

The lengths of fatty acids and their positions on the glycerol backbone determine the type of crystal formed. The triacylglycerols in a certain fat or solidified oil always form the same type of crystals unless other ingredients are added to alter crystal formation. To produce a margarine with enhanced β' stability, it is necessary to have a variety of triacylglycerols with different fatty acid chain lengths. Palm and hydrogenated cottonseed oils contain a fair amount of C16:0 and can be added to other oils to enhance β' structure.

Shortenings

Shortenings are semisolid fats that impart a "short" or tender quality to baked goods, enhance the aeration of leavened products, and promote a desirable grain and flavour. They coat the gluten proteins of flour which prevents toughness. In contrast, toughness is desirable in yeast-raised products to give a chewy texture. For products with characteristics between breads and cakes such as doughnuts, shortening modifies the gluten and adds richness to the product. In baked goods, shortenings are used specifically to leaven, cream and lubricate. In icings and fillings, the fats help to form tiny air bubbles that create a light and fluffy structure. Shortenings used as stable frying fats provide a heating medium, and their crystalline structures are not important.

The requirements of fats with properties for shortening are fairly specific depending upon the food in which they are used. Bakery shortenings should have as wide a plastic range as possible, that is, the melting behaviour should remain constant over a specified temperature range, often 24-42°C. This quality allows the fat to be easily manipulated without melting at room temperature and enhances its mixing ability. A wide plastic range is accomplished by blending a partially hydrogenated stock with a fully hydrogenated oil such as soybean (β crystal) or cottonseed and palm (β' crystal). The β' crystal is often preferred because it results in a creamier texture.

Salad oils

A major use of salad oils is in pourable salad dressings. Traditional salad dressings, some of which are emulsified, consist of a two-phase system of oil and water with 55-65 percent oil. A salad oil coats the salad ingredients, spreading the flavour of the dressing that improves the palatability of the salad. The other major use of salad oils is in mayonnaise and thick salad dressings, which contain 80 and 35-50 percent oil, respectively. The oil in mayonnaise is responsible for viscosity, whereas the oils in thick salad dressings help to modify the mouthfeel of the starch paste that thickens the product.

A salad oil must not contain solid crystals that, when refrigerated, would impart a waxy, tallowy texture, would break the emulsion formed between water and oil, or would give the product a cloudy appearance. Oils can be winterized, a process that removes solid crystals formed at refrigerator temperatures.

Typically, unhydrogenated or partially hydrogenated soybean, canola, winterized cottonseed, safflower, sunflower, and corn oils are used. Olive oil has a unique flavour, although it forms crystals at refrigerator temperature, it is often served at room temperature as a salad oil.

Medium-chain triglycerides (MCT)

In addition to the common dietary fats, lipid fractions such as medium-chain triglycerides (MCT oil) are used in specialized therapeutic preparations. MCT oil is a fraction of coconut oil containing fatty acids of 8-10 carbon atoms in triacylglycerols. MCT oil is used in formulas for enteral feeding and in diets for patients with malabsorption syndromes.

Conclusion

The range of food uses requires a variety of fats and oils with characteristics tailored to meet these needs. Efforts to modify the composition of fats and oils may be limited by these various technical requirements.

Chapter 7

Lipids in early development

Maternal nutrition

During pregnancy, the acquisition of long-chain polyunsaturated fatty acids for placental and fetal development is required. In addition, there is firm evidence that maternal nutrition and health in the periconceptional period is of crucial significance. Events prior to conception influence the long term physiological process of fat storage and the nature of the fat store. This is the fat which is available during the critical period of cell commitment and division during embryonic and placental development in the first trimester. Substantial amounts of fat are also stored from conception to provide for fetal growth in the third trimester and the initial needs of lactation.

The evidence from animal experiments suggests that pre and post-natal nutrition have pronounced effects on brain lipid composition and learning (Galli and Socini, 1983). Specific deficits of n-3 fatty acids influence neural integrity (Budowski, Leighfield and Crawford, 1987) and selectively affect learning and visual abilities (Wheeler, Benolken and Anderson, 1975; Lamptey and Walker, 1976; Bourre *et al.*, 1989; Yamamoto *et al.*, 1987). Studies with non-human primates confirm that n-3 deficiency depresses the development of retinal function and visual acuity (Neuringer, Anderson and Connor, 1988; Connor, Lin and Neuringer, 1990).

Recent studies replicated these findings in human infants indicating the essentiality of n-3 fatty acids and the need for the inclusion of docosahexaenoic acid (DHA) in food for infants (Birch *et al.*, 1992, 1993 a, b; Carlson *et al.*, 1993 a, b, Uauy *et al.*, 1990; Uauy, Birch and Birch, 1992). Although there are not similar studies for arachidonic acid, the experimental evidence indicates that low arachidonic acid levels are associated with low pre-natal (Crawford *et al.*, 1989; Leaf *et al.*, 1992) and post-natal growth in preterm infants (Carlson *et al.*, 1992). Conditionally, arachidonic acid should be considered as an essential nutrient during early development because: it is present in human milk (Sas *et al.*, 1986; Koletzko, Thiel and Abiodun, 1992) along with DHA; the interplay between the n-3 and n-6 families; the specific functions of arachidonic acid in neural and vascular function; and the role of its eicosanoids in cell regulation.

There have not been the same kind of controlled, randomized trials for term infants as those performed for preterm ones. However, the preliminary studies comparing term infants who were breastfed with those fed low α-linolenic acid formula suggest that the term infant's brain may also be responsive to external nutritional influences (Birch *et al.*, 1993 b; Gibson *et al.*, 1993). Data on brain composition provide a comparison of formula-fed versus breast-milk-fed babies and give further evidence that dietary fatty acids influence the developing brain of term infants (Farquharson *et al.*, 1992).

Preconceptional nutrition

Embryology and clinical studies provide evidence that the mother's nutritional status around the time of conception has greater significance in terms of birth weight (Caan *et al.*, 1978; Villar and Riveria, 1988; Wynn *et al.*, 1991), prevention of neural tube defects (Wald *et al.*, 1991) and non-genetic congenital disorders (Wynn and Wynn, 1981) than nutritional status in the latter part of pregnancy. Poor maternal nutrition or metabolic status at this early stage represents a significant risk of compromising embryonic development, cell commitment and the rate of DNA replication in a manner which cannot be compensated later.

During gestation, the placenta selects arachidonic acid and docosahexaenoic acid (DHA) at the expense of linoleic acid, α-linolenic acid and eicosapentaenoic acid (EPA) resulting in substantially higher proportions of arachidonic acid and DHA in the fetal circulation at mid-term (Crawford *et al.*, 1976) and term (Olegard and Svennerholm, 1970). This is denied to babies born prematurely. Low birth weight and prematurity are associated with a high risk of neurodevelopmental disorders and disabilities (Wynn and Wynn, 1981; Dunn, 1986; Hack *et al.*, 1991; Scottish Low Birth Weight Study Group, 1992 a, b). The incidence of neurodevelopmental disorder among premature and low birth weight babies in the UK and Sweden is reported as having increased threefold since 1967 (Pharoah *et al.*, 1990; Hagberg, Hagberg and Zetterstrom, 1989) adding urgency to the need for a better understanding of the nutritional needs of these infants.

Low birth weight. In a number of developing countries, low birth weight is a particular problem associated with a high incidence of perinatal and maternal mortality and morbidity. It is also a problem within urban areas of developed nations, especially among lower socio-economic groups. Worldwide, WHO estimates that 17.4 percent of babies are born at low birth weights. This has important long term implications for the children's health and abilities (FAO/WHO, 1992).

Teenage pregnancies pose a special problem since the nutritional intake of the mother needs to support her own continued somatic growth as well as that of the fetus. The vulnerability of teenage mothers and their offspring is borne out by the high frequency of perinatal mortality, low birth weight and maternal morbidity and mortality. However, when pregnant teenagers eat adequately throughout their pregnancies, delivery outcomes may be successful.

While a relationship exists between maternal nutrition and birth weights (Caan *et al.*, 1978), the causes of low birth weight are multifactorial, including: low caloric intake, low weight gain during pregnancy, low prepregnancy weight, short stature and disease (for example, malaria) (FAO/WHO, 1992). In developed countries, smoking presents an additional factor. Several nutrients, independent of smoking, are related to birth weight up to 3.0 kg (Doyle *et al.*, 1990). Retrospective evidence suggests that fetal nutrition is a determinant of risk of non-insulin-dependent diabetes and vascular disease in later life (Barker *et al.*, 1993). This concept is supported by evidence of deficits of arachidonic acid, DHA and vitamin A in the circulation of low-birth weight newborns (Ongari *et al.*, 1984; Crawford *et al.*, 1989, 1990; Carlson *et al.*, 1992; Leaf *et al.*, 1992) and by evidence of vascular pathology in the placenta of low birth weight babies (Althabe, Laberre and Telenta, 1985; Winick, 1983).

Micronutrients and fat store. In many developing countries, vitamin A deficiency is still prevalent. An adequate intake of fat and fat soluble vitamins under these circumstances is important and there should be an effort to raise the percentage of dietary energy from fat to at least 20 percent for women of reproductive age. This strategy should also help to ensure an adequate supply of essential fatty acids and fat soluble vitamins.

Body fat plays a special role insofar as women on low fat and low-calorie diets and highly trained athletes may not conceive, or the embryonic and fetal development of their offspring may be jeopardized (Frisch, 1977). The mother's fat store at the time of conception will be relevant to maternal hormonal responses and to the nourishment of the embryo. Similarly, it will provide the basis for subsequent fat storage and utilization during the pregnancy (FAO/WHO, 1978). To ensure adequate nutritional preparation, women should not be on slimming, low calorie or low fat diets in the three months prior to conception. Calorie and nutrient intakes should be adequate to meet the general recommendations for the first trimester of pregnancy whilst essential fatty acid intakes should be similar to those mentioned below for the pregnancy itself to ensure an adequate quality of the fat store. The need for adequate nutritional status in terms of calories, vitamins, minerals and trace elements before conception should receive attention.

Pregnancy. An additional requirement for dietary fat is present throughout the nine months of pregnancy to provide for the fat storage in the early trimester and the growth in other compartments during the later trimesters. In the first trimester, the development of the embryo imposes a negligible quantitative demand for additional essential fatty acids, however, normal maternal fat deposition and uterine growth as well as the preparative development of the mammary gland do make significant demands. In the second and particularly the third trimester, the expansion of blood volume, placental and fetal growth add to the demand. Based on accretion data for these compartments, an average total of 600 g of essential fatty acids (approximately 2.2 g/day) will be acquired throughout a normal pregnancy of a well-nourished woman. Increased energy utilization may modify this need. This is consistent with the recommendation on increased energy requirements (WHO, 1985a) and will maintain the linoleic to α-linolenic balance with a ratio of 1:5 to 1:10.

This recommendation presumes adequate conversion of both parent essential fatty acids to their long-chain polyunsaturated fatty acid derivatives. However, recent studies suggest that a relative deficiency of long-chain polyunsaturated n-3 fatty acids develops during pregnancy (Holman, Johnson and Ogburn, 1991). Correlations between arachidonic acid and birth weight and between DHA and gestational age (Leaf *et al.*, 1992) are consistent for either indicator of maturation and with DHA intakes. There is evidence that high fish intakes are associated with longer gestation, higher birth weights and a reduced incidence of premature births. An intervention study with fish oils indicates that long-chain polyunsaturated n-3 fatty acids play a role (Olsen *et al.*, 1992). If these data are confirmed, this could indicate the need to consider DHA preformed to help prevent premature birth and pregnancy-related hypertension (WHO, 1985a).

Lactation. During lactation, there are increased nutrient and energy requirements. The additional energy requirement for lactation, assuming there has been an appropriate energy store laid down during pregnancy, is 500 kcals/day (WHO, 1985a). If the stores are limited, the energy need can be as high as 800 kcals/day. Fat output is the most variable component of milk and this depends, qualitatively and quantitatively, on maternal nutrition and prolactin secretion. The diet of a well-nourished mother provides the unsaturated fatty acids within a range that assures the essential fatty acid supply to the infant. The overall energy may be supplied by any source but the essential fatty acid component is fully dependent on fat in the diet and maternal stores. If a woman was well-nourished throughout pregnancy, her fat stores can provide approximately one-third of the energy and essential fatty acids needed for the first 3 months of lactation. Based on the composition of milk from omnivorous women, the mother's diet during lactation should provide an additional 3 to 4 g/day of essential fatty acids in the first trimester of lactation which should rise to 5 g/day as the fat stores become depleted (Koletzko, Thiel and Abiodun, 1992) (Table 7.1). It should be possible to do this by increasing normal food intake.

Infant nutrition

The premature infant. During the last decade, evidence on the accretion of parent essential fatty acids and derived long-chain polyunsaturated fatty acids for fetal and neonatal growth has become available (Martinez, 1988). Modern analytical techniques have provided

TABLE 7.1
Average fatty acid values of mature human milk in Europe and Africa

	Medians and Ranges	
	Europe (14 studies)	Africa (10 studies)
Fatty acid totals (% wt/wt)		
Saturated	45.2 (39.0-51.3)	53.5 (35.5-62.3)
Monounsaturated	38.8 (34.2-44.9	28.2 (22.8-49.0)
n-6 + n-3 PUFA	13.6 (8.5-19.6)	16.6 (6.3-24.7)
n-6 PUFA (% wt/wt)		
C18:2n-6	11.0 (6.9-16.4)	12.0 (5.7-17.2)
C20:2n-6	0.3 (0.2-0.5)	0.3 (0.3-0.8)
C20:3n-6	0.3 (0.2-0.7)	0.4 (0.2-0.5)
C20:4n-6	0.5 (0.2-1.2)	0.6 (0.3-1.0)
C22:4n-6	0.1 (0.1-0.2)	0.1 (0.0-0.1)
C22:5n-6	0.1 (0.0-0.2)	0.1 (0.1-0.3)
Total n-6 LCP	1.2 (0.4-2.2)	1.5 (0.9-2.0)
n-3 PUFA (%wt/wt)		
C18:3n-3	0.9 (0.7-1.3)	0.8 (0.1-1.44)
C20:5n-3	0.2 (0.0-0.6)	0.1 (0.1-0.5)
C22:5n-3	0.2 (0.1-0.5)	0.2 (0.1-0.4)
C22:6n-3	0.3 (0.1-0.6)	0.3 (0.1-0.9)
Total n-3 LCP	0.6 (0.3-1.8)	0.6 (0.3-2.9)

Source: Adapted from Koletzko, Thiel and Abiodun, 1992.

substantial data on the fatty acid composition of human milk (*Ibid.*). Babies born prematurely are denied the intra-uterine supply of arachidonic acid and DHA. In addition, they are born with little or no fat reserves making them fully dependent on the diet. Despite the provision of ample linoleic acid and rising linoleic acid levels in the plasma, it is evident that arachidonic acid and other polyunsaturated fatty acids (of at least 20 carbon atoms in length) drop rapidly (Leaf *et al.*, 1992). Present assessment of essential fatty acid status is dependent on the measurement of plasma and red cell composition. While there are limited data on tissue pools, the primate data suggest that they do correlate (Neuringer, Anderson and Connor, 1988). Retinal and visual cortical function show the effects of tissue essential fatty acid status.

Several studies on biochemical indices of essential fatty acid status documented the effect of adding long-chain polyunsaturated fatty acids to the diet (Koletzko *et al.*, 1989; Carlson *et al.*, 1992; Uauy, Birch and Birch, 1992; Makrides *et al.*, 1993). If both arachidonic acid and DHA are added, the post-natal drop in blood plasma level can be ameliorated. If marine oil supplementation is used (EPA/DHA = 2:1) this compromises the level of arachidonic acid which was found to adversely affect growth in one study (Carlson *et al.*, 1992). There is convincing evidence that supplementation with DHA from marine oil improves the development of the rod photoreceptor and visual acuity, a measure of the receptor response to light, as well as the cortical, which is associated with cognitive ability to integrate information (Uauy *et al.*, 1990; Uauy, Birch and Birch, 1992; Birch *et al.* 1992; Carlson 1993a). The initial concern (Carlson *et al.*, 1992) that poor growth, associated with marine oil, was linked to depressed arachidonic acid status has been removed by using a low EPA marine oil with an EPA/DHA ratio of 1:10. This did not compromise weight gain and resulted in higher Bayley mental development scores at 12 months (Carlson *et al.*, 1993 b). Eight year follow-up studies which compared premature babies who were fed human milk by tube with those fed formula showed an IQ which was eight points lower in those children who had been fed formula (Lucas *et al.*, 1992).

There are limited data on the accretion of essential fatty acids of normal fetuses and healthy infants fed human milk and no quantitative human data on the metabolic conversions. Although there are variations in maternal diets, milk composition data obtained from omnivorous women were the basis for the consultation's recommendation. As a guide, formula for preterm babies should provide a mean of 700 mg linoleic acid, 50 mg α-linolenic acid, 60 mg of arachidonic acid and its associated long chain n-6 fatty acids, and 40 mg of DHA per kg body weight. That is, 5.6 percent of the energy as parent essential fatty acids and 0.8 percent as long-chain polyunsaturated fatty acids. Because of the known interference of linoleic acid with the metabolism of the long-chain polyunsaturated fatty acids, linoleic acid should not exceed 10 percent of the total energy. Until more data are available, particularly dose response data, the other fatty acids as mentioned above should remain within approximately 30 percent of the target recommendation. The remaining fatty acids should be selected on the basis of digestibility, lack of interference with essential fatty acid metabolism and other measures of function. In principle, a mixture of oleic and saturated fatty acids with a predominance of oleic appears to meet these conditions.

Term infants. Whenever possible, the preferred source of infant nutrition is human milk. In view of the evidence, education and health care programmes should actively promote breast feeding. In addition, because of the long term nutritional interactions with the mother even prior to conception and their profound implications for public health, similar programmes should be developed for women prior to conception, through pregnancy and lactation. Such programmes will improve maternal health, fetal development and neonatal nutrition.

When term infants who died from unexplained causes were compared, those infants who were fed milk formula without arachidonic acid and DHA had reduced levels of brain cortical DHA and higher n-6 docosapentaenoic acid (an index of DHA deficiency) than those fed breast milk (Farquharson *et al.*, 1992). As the data on preterm infants now demonstrate, there are advantages for both rod photoreceptor and neural function to supplementing with n-3 long-chain polyunsaturated fatty acids; it would seem proper to provide both arachidonic acid and DHA preformed in term infant formula milks in similar proportions to breast milk from well-nourished, omnivorous mothers. For term infants, the provision, per kilogramme of body weight should amount to 600 mg of linoleic acid, 50 mg of α-linolenic acid, 40 mg of arachidonic acid and its associated n-6 fatty acids and 20 mg of docosahexanoic acid. Although there is not yet evidence from controlled, randomized trials in term infants, this is suggested to provide for the greatest possible release of the full genetic potential for neural and visual development.

As it has evolved, human milk provides long-chain polyunsaturated fatty acids preformed. In view of the evidence on the higher efficiency of long-chain polyunsaturated fatty acids for neural development (Sinclair, 1975; FAO/WHO, 1978; Leyton, Drury, and Crawford, 1987) and the data on premature infants discussed above, the long-chain polyunsaturated fatty acids should be included in infant formula. Providing parent essential fatty acid at a ratio which is claimed to optimize conversion to long-chain polyunsaturated fatty acids should be proven valid for physical, vascular and mental development and should provide for biochemical and functional normalcy. The burden of proof should be placed on those proposing that artificial formula should, in principle, be dissimilar to breast milk (Drury and Crawford, 1990).

These recommendations for term and preterm infants are broadly in agreement with those of the British Nutrition Task Force on Unsaturated Fatty Acids (BNF, 1992).

***Weaning*.** Human milk provides 50 - 60 percent of its energy as lipid in which about 5 percent of the energy is essential fatty acid with 1 percent as long-chain polyunsaturated fatty acids. Six-month follow-up studies of lactation in a randomised trial showed that in well-nourished mothers, milk fat contribution increased from 40 - 50 g/litre at three weeks to 60 - 70 g/litre at 4-6 months (WHO, 1985b; Sas *et al.*, 1986). In developing countries with lower energy intakes, the rise in milk fat content was less. It would, therefore, seem especially inappropriate to wean babies on to low fat diets.

The European Society of Paediatric Gastroenterology and Nutrition (1991) recommended that 40-55 percent of the dietary energy be provided as fat (4.4-6.0 g/100 kcal) for follow-up formula. During weaning, the fat component should provide 30-40 percent of the dietary energy and similar levels of essential fatty acids as are found in breast milk from appropriate foods until at least two years of age.

In practice, this means that complementary food used during the weaning period should include adequate amounts of fats and oils as the breast milk component of the diet declines. Commonly, infants in many developing countries are weaned on to cereal or tuber-based diets with a low energy density. In these countries, there is a need to reinforce educational messages about the use of vegetable oils or oil containing foods in the diet of weanling infants and young children.

Sources

It is acknowledged that human milk is the best and only time-proven source for fat and long-chain polyunsaturated fatty acids in neonate diets. Sources of long-chain polyunsaturated fatty acids that have been tested include egg phosphoglycerides, fish oils and fish oil fractions. Novel sources of fat which have been developed include phosphoglycerides from animal tissues, algal and microbial lipids. The safety and efficacy of novel sources should be adequately tested before they are added to infant formula.

Chapter 8

Health, obesity and energy values of dietary fat

Overweight is defined as an increase above a standard in body weight related to height. It is often measured by the Quételet or Body Mass Index (BMI) which is weight in kilograms divided by the square of stature in metres (BMI = weight/height^2). Obesity is also defined as an abnormally high percentage of body fat. In males, normal body fat represents 12-20 percent of body weight. Body fat represents 20-30 percent of body weight in normal females.

Weight standards for a population can be established by dividing a large sample of the population according to the normal distribution of their body weights in relation to height. The inherent difficulty with this approach is that there is an underlying assumption that average weights are healthy weights. An alternative approach to determining standards for healthy weights is to use body weights which are associated with the lowest overall risk to health. In several prospective studies, the lowest death rate was associated with a BMI close to 22 (Manson *et al.*, 1987; Garrison and Kannel, 1993).

Longitudinal tracking of weight. Several epidemiological studies have examined the weight status of various populations over time (Noppa and Hallstrom, 1981; Borkan *et al.*, 1986; Williamson *et al.*, 1990) and they show a progressive rise in BMI in most populations. However, the weight status of an individual from infancy and childhood through adolescence and into adult life often follows an uneven track. (Bradden *et al.*, 1986; Charney *et al.*, 1976; Khoury *et al.*, 1983; Mossberg, 1989; Zack *et al.*, 1979).

A few studies (Garn *et al.*, 1986; Johnston and Mack, 1978; Melbin and Vuille, 1976) have calculated the relative risk of being in the top weight category as an adult based on weight status in childhood or adolescence. When the lightest youngsters were compared with the heaviest ones, the heaviest youngsters were 1.6 to 2.5 times more likely to be overweight as adults. However, the Harvard Longitudinal Studies of Child Health and Development 50 year investigation found that the BMI of females during childhood had essentially no correlation with their BMIs when they reached middle-age (Casey *et al.*, 1992). There was a better correlation between the BMIs of adolescent females and those of women at age 50 years, although this was still low. In females, the failure to show an effect of overweight in adolescence on mortality in adults may be due to this low correlation. In men, on the other hand, the correlation of BMI in childhood or adolescence with BMI at age 50 years was better, but the tracking of childhood and adolescent weight into adult life is still of a low order (Borkan *et al.*, 1986).

Children from families where one or both parents are overweight are at higher risk of adult obesity. If these children are notably obese during their school years, a preventive programme may be advisable. Thus, ongoing surveillance of children and adolescents will help identify those who are at risk and may be helped by preventive service.

Health risks associated with obesity

Overweight and fat distribution are useful predictors of premature mortality and the risks of heart disease, hypertension, non-insulin-dependent diabetes mellitus, gallbladder disease and some cancers. However, if body fat *per se* were the major risk factor associated with premature mortality, obese women might be expected to have shorter life expectancies than obese men. This is generally not the case, and it is now recognized that it is fat distribution, especially an increase in abdominal and visceral fat, that is a predictor of the health risk related to obesity. For example, weight gain of less than 5 kg in women during adult life may carry little extra risk, particularly if the added weight is located in the femoral region. For most men, any weight gain after the age of 20 years increases risk because this fat is usually deposited as abdominal and visceral fat.

In epidemiology, the relationship between data on BMI and a given disease risk is shown to be curvilinear and this curve is often described as J- or U- shaped. This means that mortality and morbidity tend to increase as the BMI increases above 25 or drops below 18.5.

Life insurance statistics from the United States show that excess weight is associated with higher mortality rates. Based on 1979 data (Society of Actuaries and Association of Life Insurance and Medical Directors of America, 1980), a body weight which is 10 percent above average weight is accompanied by an 11 percent increase in excess mortality for men and a 7 percent increase for women. If body weight is 20 percent above average, the excess mortality rises to 20 percent for men and 10 percent for women. Conversely, the minimum mortality rates for both men and women occur among individuals who are approximately 10 percent below average weight.

More than six studies have been published showing that central adiposity is positively correlated with increased mortality as well as the risk for developing cardiovascular disease, diabetes mellitus and stroke (Lapidus *et al.*, 1984; Larsson *et al.*, 1984; Donahue *et al.*, 1987; Ducimetiere, Richard and Cambien, 1986; Stokes, Garrison and Kannel, 1985). Among the highest quintile for central body fat, the relative risk of myocardial infarction was 8.2 times higher than that for the lowest quintile. For stroke and mortality in general, the relative risk was 3.8 and 2.8 times higher for individuals in the highest quintile compared to those in the lowest quintile.

Losing and regaining weight, so-called weight cycling, may also be hazardous. Data from the Chicago Gas and Electric Company Study (Hamm, Shekelle and Stamler, 1989) showed that those people who gained and lost weight had a significantly higher risk of death from cardiovascular disease than the group of individuals with no change in weight. More recently, data from the Framingham study were used to show that significant changes in weight were associated with a higher likelihood of mortality (Lissner *et al.*, 1991). Caution in interpreting data on weight cycling as a cause of detrimental changes is urged.

Obesity must modify some intermediate mechanism such as cardiac function, the metabolism of lipids or glucose to produce death or disease. However, even though severe overweight generally increases the risk of death, especially sudden death, in many studies it could not be seen as an independent variable. Two major problems plague the interpretation of studies in terms of obesity as an independent factor (Manson *et al.*, 1987). First, many studies fail to separate smokers from non-smokers. Since smokers as a group tend to have lower body weights and higher mortality rates, including them in a study population influences the death rates and confounds the assignment of effects to body weight *per se*. Second, early mortality may bias the interpretation of weight status on life expectancy. For example, individuals who have lost or are losing weight at the time of an initial survey may die and thus overemphasise the effect of low body weights as a cause of higher mortality. The failure to identify obesity as an independent risk factor has led many to suggest that it is unimportant.

Diabetes mellitus. More than 90 percent of all diabetics have non-insulin dependent diabetes mellitus (NIDDM), and excessive weight gain and overweight are the major nutritional factors which increase the risk of developing NIDDM. The importance of body weight is demonstrated by the low level of NIDDM during World War II and during periods of famine. NIDDM is almost non-existent in individuals with a BMI of 20 or less. A BMI of 35 has an 8-fold increase in risk of NIDDM when compared with a BMI of 25.

Other types of diabetes mellitus are insulin-dependent (IDDM), gestational diabetes and a very rare form, malnutrition-related diabetes mellitus. IDDM is an immunologic disease which destroys the insulin-producing cells. Patients with this disease require insulin for survival. The nature of the autoimmune stimulus to pancreatic destruction is unknown. The role of diet in the development of this disease is not established.

The San Luis Valley study in the USA provided some evidence to support the hypothesis that a high fat, low-carbohydrate diet increases the risk for onset of non-insulin-dependent diabetes (Marshall, Hamman and Baxter, 1991). This involved cross-sectional analyses of individuals without a prior history of diabetes. A 24-hour diet recall preceded a glucose tolerance test. A high intake of fat appeared to be associated with impaired glucose tolerance, however, this may be the result of weight gain.

Other evidence comes from groups who changed their dietary patterns. These studies suggest that higher intakes of dietary fat and/or the weight gain which is associated with such increases, may be a factor in the onset of non-insulin-dependent diabetes. The Australian Aborigines were prone to the disease when they made a transition from their traditional manner of living to an urban life-style (O'Dea, White and Sinclair, 1988). The mild elevation in plasma triglycerides and fasting insulin levels were consistent with insulin resistance. A group of Bangladeshis who had emigrated to the UK were found to have low plasma cholesterol concentrations, enhanced insulin resistance and three times the usual prevalence of diabetes, as well as a high rate of morbidity and mortality from coronary heart disease (McKeigue *et al.*, 1988). A deterioration in carbohydrate tolerance and changes in lipoprotein patterns of the Pima Indians and Caucasians also point toward problems with the modern, high fat diet and obesity (Swinburn *et al.*, 1991).

Ethnic differences in the C-peptide response relative to the insulin response in testing for glucose tolerance led to the tentative conclusion that dietary fat may be involved in determining insulin secretion and its clearance in the liver (Cruickshank *et al.*, 1991). There is still more speculation than evidence to link dietary fat with non-insulin-dependent diabetes. At the present time, the general rationale for modifying dietary fat intakes has been to reduce the risk of coronary heart disease in diabetics.

Causes of obesity

Nutrient balance of fat storage. Obesity is always a chronic failure to balance nutrient intakes with expenditure (oxidation) (Bray, York and Fisler, 1989). There are various causes of obesity. At one extreme, obesity may be due simply to excess food (energy) intake in relation to energy requirements. In these cases, factors of heredity play an important role in the genesis of obesity which can develop even when the diet is composed primarily of carbohydrates. At the other extreme, there are those types of obesity where dietary composition, particularly a high fat intake, is central to its development. Any of these types of dietary obesity can be controlled by changing the composition of diets, by restraining the intake of food, or by increasing nutrient oxidation.

While overall energy balance is central to obesity, the concept of macronutrient balance is also useful for understanding the factors affecting excess weight gain and loss. In a normal adult, the daily energy intake from carbohydrates would be between 50 and 100 percent of the total carbohydrate stores, in the body. In contrast, the protein intake would be a little over 1 percent of total stores while the fat intake would be considerably less than 1 percent of that stored in the body. Glycogen (the storage form of carbohydrate) metabolism is closely regulated and, depending on the balance between carbohydrate intake and oxidation, there can be wide fluctuations in the carbohydrate stores over even short periods of time. This is not the case with protein and fat stores for which it takes considerably longer to affect appreciable change.

The process of regulating nutrient balance is complex. The feedback model for nutrient balance is described as having four components (Bray, 1987). The first is the "controlled system" which consists of the intake, digestion, absorption, storage and metabolism of the nutrients in food. The second is the "controller" located in the brain, and the third is comprised of the feedback signals which tell the controller about the state of the controlled system. Finally, there are the mechanisms that modulate nutrient intake and energy expenditure.

In general, there are effective feedback and control mechanisms for regulating and balancing carbohydrate intake and oxidation (Flatt, 1988). It is more difficult to become obese by eating a very high carbohydrate diet than by eating a high fat diet for a number of reasons. First, the quantity (volume) of high carbohydrate/high fibre foods which is required is much larger than that needed with a high fat diet. Second, the storage capacity for carbohydrates is limited. Third, the biochemical pathways for conversion of carbohydrate to fat are limiting and energetically expensive and are virtually negligible under normal dietary conditions in humans. Finally, the intake of carbohydrate stimulates carbohydrate oxidation so that carbohydrate balance is maintained once glycogen stores are filled, a process also stimulated by carbohydrate consumption.

Protein balance is also well-controlled. Protein stores rise gradually and only in response to stimuli other than increased protein intake. Protein which is consumed in excess of what is needed for tissue building and repair as well as enzyme formation, is converted to carbohydrate. Positive protein balance can contribute to overall energy balance in the same way that positive carbohydrate balance does.

Chronic imbalance between fat intake and fat oxidation can lead to changes in fat stores of adipose tissues. Avoiding storage of the fat consumed in a high fat diet requires that the dietary fats be oxidized. In clinical studies low rates of fat oxidation in the basal state predict an increase in body weight (Zurlo *et al.*, 1990). It is only when the oxidation of fat equals the intake of fat that a stable body weight can be achieved.

While carbohydrate and protein oxidation are affected by carbohydrate and protein consumption, fat oxidation is not affected by fat intake and the day-to-day relationship between fat balance and fat intake is weak (Flatt, 1988). Fat oxidation is related most strongly to energy balance (with a negative energy balance promoting fat oxidation) and also to the degree of body fat (Schutz *et al.*, 1992; Zurlo *et al.*, 1990). Creating a negative energy balance through exercise or dietary restriction can effectively increase fat oxidation, as can reducing the fat content of the diet. However, as weight is lost, fat oxidation also tends to decline. To prevent an individual from regaining weight which has been lost, the intake of fat must be reduced by approximately 20 g/day for each 10 kg of fat lost (Schutz *et al.*, 1992).

There are major differences between individuals in their capacity to increase the oxidation of fat after beginning a high fat diet (Zurlo *et al.*, 1990). While much of this difference is genetic, physical training can increase oxidation of fatty acids by muscle and reduce the tendency to gain weight. For this reason, adequate physical activity should be part of any weight control programme.

Some papers have shown a weak positive correlation between fat intake and body weight (WHO, 1990; Romieu *et al.*, 1988, Miller *et al.*, 1990) but others have not. Access to a diet with more than 30 percent energy from fat produces obesity in many animals. It was found that women who have lowered fat intakes have reduced body weight providing further evidence of a positive association between fat intake and body weight. However, in longer trials the effect has been small, probably because compliance during long studies fails (Sheppard, Kristal and Kushi, 1991; Lissner *et al.*, 1991, Lee-Han *et al.*, 1988).

Diet Caloric Density. It is well-recognized that energy per 100 grams of diet, referred to as caloric density, increases as fat content rises. Low-fat diets have resulted in weight loss in short-term studies, however, in long-term trials, low-fat diets in premenopausal women resulted in the consumption of an additional 19 percent of energy to maintain weight (Prewitt, 1991). The real long-term change is unknown because food was supplied to the subjects in these studies. In free-living persons, reinforcement and motivation are needed for adherence to a low-fat diet.

From the data available, it is clear that obesity is a chronic problem for which the cure is very elusive. Weight loss usually occurs during effective treatment; however, when treatment is terminated weight regain is common. This is consistent with the course of most chronic problems, and clearly indicates that prevention is the preferred method of controlling obesity.

Energy values of fat

Fatty acids are the dietary components that account for the highest amount of energy liberated during oxidation of lipids. Glycerol, to which most fatty acids are esterified, is a 3-carbon molecule with 3 oxygen atoms. Although glycerol is 10 percent by weight of triacylglycerol, it accounts for only 5 percent of the energy. The fatty acids of food impart the high energy value that is of nutritional interest.

The traditional factor for calculating the amount of dietary fat is 9 kcal (37.7 kj)/g compared to 4 kcal (16.7 kj)/g for carbohydrate and protein (FAO/WHO, 1978). Originally these values were proposed by Atwater, and are based on the amounts of energy liberated when these macronutrients are metabolically oxidized, allowing for incomplete intestinal absorption.

The factor of 8.37 has been used for many decades to convert grams of fat in cereals, fruits and vegetables to calories (Merrill and Watt, 1955). The precision in this 3-digit number is unwarranted, particularly upon examination of its derivation.

At the turn of the century, Atwater proposed factors for estimating the energy value of nutrients. The heat of combustion for triglycerides was applied to extracts of various foods, but for cereals, fruits and vegetables the "assumed or calculated" value was given as 9.30 calories per gram. Merrill and Watt (1955) copied this figure from a table created in 1900 by Atwater and Bryant. Merrill and Watt wrote: "For fat as it occurs in cereals and other plant sources Atwater assumed the apparent digestibility to be 90 percent and we have continued this practice. The energy factor for fat in plant foods is therefore, assumed to be 8.37 calories per gram."

Since Atwater had used a coefficient of digestibility of 95 percent for butter, it was assigned also to the separated fats of plant origin. The calculation for fats and oils as such became 95 percent of 9.30 or 8.84 calories per gram. These factors have a long history of many assumptions. The corrections for digestibility are inappropriate for the dietary oils and fats commonly consumed, and the factor of 9 for converting grams of all dietary fat to calories is more suitable and offers consistency.

Fat substitutes

Advice to reduce fat and energy consumption has led to the production of foods with a lower content of fat and to the development of fat substitutes. There are two principal approaches to the replacement of dietary fat. The first involves hydratable carbohydrates and proteins with the mouthfeel of fats. The second includes non-absorbed synthetic substances with the physical properties and technical function of fat within foods.

Protein and carbohydrate materials can be produced in forms that binds up to three times their weight in water and that create particles that imitate the mouthfeel of fat. When the hydrated material replaces fat in food, the caloric reduction is from 9 kcal/g to 1 to 2 kcal/g. The carbohydrates used are the low molecular weight starches, dextrins, maltodextrins and gums (xanthan for example), the proteins for fat replacers come primarily from milk and egg white. Since these substances have not been altered chemically, they are digested, absorbed and metabolized like ordinary nutrients (Vanderveen and Glinsmann, 1992).

The other group of fat substitutes, which has not yet reached the market, is produced by chemical synthesis from a wide range of possible structures having different digestibilities and effects on gastrointestinal function. Such material replaces fat on a gram to gram basis. The concern about some non-absorbable lipophilic substances such as fatty acid esters of sugars revolves around impaired absorption of fat-soluble compounds, both vitamins and drugs. For example, the prolonged use of sucrose polyester had a negative effect on the body stores of d-tocopherol (Jandacek, 1991). Individual assessments for safety are required for each type of synthesized fat substitute (Borzelleca, 1992; Vanderveen and Glinsmann, 1992).

The value of these kinds of materials for reducing either the total energy consumption, as an aid in the treatment or prevention of obesity, or as modifiers of a kind of dietary fat remains to be investigated. The assessment of fat substitutes must include the effect on the total diet and the redistribution of macro- and micronutrients (Rolls and Shide, 1992). Extrapolation from animal studies may be inappropriate since human beings can choose replacements from a wide variety of foods.

Special attention must be given to the effect of fat substitutes on the consumption of micronutrients, such as vitamins A, D and E, since certain dietary fats are often important sources of these nutrients and the amount of dietary fat may be important in the utilization of such materials.

Conclusions

Obesity and excess visceral fat are conditions that predispose individuals to chronic diseases. Excess body fat is a major risk-factor of morbidity and mortality in most affluent countries and, increasingly, in many developing countries.

High intakes of dietary fat are associated with increasing levels of obesity in animals. For humans, the relationship between the rates of fat intake and oxidation is clear, however, the association between fat intake as a proportion of energy and obesity is weak.

Prevention of obesity involves early recognition and surveillance, regular physical activity and, for adults, a restrained pattern of eating.

Chapter 9

Coronary heart disease and lipoproteins

Coronary Heart Disease (CHD), characterized by a limited supply of oxygen to the heart muscle, has clinical manifestations ranging from angina pectoris to myocardial infarction (MI) and sudden death. The primary cause of CHD is coronary atherosclerosis (ATS), due to lipid-rich lesions in the intima of the coronary arteries. These begin in early life as "fatty streaks" and later form fibrous, often calcified and ulcerated, lesions that narrow the arterial lumen. A thrombus, superimposed on the lesions, may precipitate MI and sudden death. These events depend upon the atherosclerotic lesion and a complex interplay of haemostatic factors. While the mechanisms are only beginning to be elucidated, it is apparent that the processes leading to CHD involve the development of ATS, thrombosis and vascular reactivity as well as their interrelationships.

Epidemiology and experimental evidence

Since the 1940s and 1950s, studies within populations as well as cross- cultural comparisons produced abundant evidence that higher serum cholesterol levels are associated with increased risk of CHD (Levy *et al.*, 1979; Anderson, Castelli and Levy, 1987; Committee on Diet and Health, 1989; Pooling Project, 1978). Recent studies, such as the Multiple Risk Factor Intervention Trial (Stamler, Wentworth and Neston, 1986) in the USA showed a continuous, graded, statistically significant association between baseline serum cholesterol and six-year, age-adjusted CHD death rates for both hypertensive and normotensive individuals and for both smokers and non-smokers. A threshold level, below which an increase in serum cholesterol has no effect on risk, could not be identified. Similarly, in a Chinese population with generally low serum cholesterol levels, a statistically significant relationship between serum cholesterol and CHD mortality was found (Chen, *et al.*, 1991) indicating that any increase in serum cholesterol increases risk of CHD. Cross-country studies, such as the Seven Countries Study (Keys *et al.*, 1986; Keys, 1970) also showed a graded increase in risk of CHD as serum cholesterol levels rise. These associations between serum cholesterol levels are primarily related to levels of low density lipoprotein (LDL), the primary carrier of cholesterol in the serum.

Associations between diet, serum cholesterol levels and risk of CHD have been well-documented in cross-country comparisons (Keys *et al.*, 1986; Levy *et al.*, 1979; Committee on Diet and Health, 1989; Lewis *et al.*, 1978). Populations with relatively high intakes of fat, especially animal fat and cholesterol, have relatively high serum cholesterol levels and high mortality rates from CHD when compared with populations consuming low-fat diets.

Numerous studies on people who immigrated from low-risk to high-risk populations (Nichaman *et al.*, 1975; Halfon *et al.*, 1982; Kato *et al.*, 1973) demonstrated that environmental factors rather than genetic susceptibility led to these differences and that diet consistently played a primary role. Generally, these high-risk populations consume diets of affluent societies. It is recognized that these populations differ from low-risk populations in many ways. For example, diets of low risk populations are not only low in fat, they are commonly higher in dietary fibre and other components of plant origin. Also, individuals in such societies are less sedentary. Abundant experimental data, however, in both animals and humans confirm that there is a primary role for dietary fat and cholesterol in the control of serum lipid levels.

Comparisons of diet and serum cholesterol levels within population groups where diet is relatively homogeneous, may or may not show a correlation between dietary fat consumption and serum cholesterol levels (Morris, Marr and Clayton, 1977; Garcia-Palmieri *et al.*, 1980; Jacobs, Anderson and Blackburn, 1979). It is well-known that individuals vary greatly in serum cholesterol level, even when diet is constant (Keys, Anderson and Grande, 1959) and there are "hyper- and hypo- responders" when dietary fat and cholesterol are modified (Katan and Beynen, 1987; Katan *et al.*, 1988). In addition, the methods for estimating the intake and composition of food consumed by individuals are severely limited and often unreliable (Jacobs, Anderson and Blackburn, 1979; Livingstone *et al.*, 1990; Schoeller, 1990; Black *et al.*, 1993). Hence, it is not surprising that some studies within a population show little association between serum lipids and estimates of the fat in the diet. Intervention studies in which diet can be controlled demonstrate that marked changes in serum lipids can be induced by changes in fat and cholesterol intake, thus, they are consistent with the epidemiological findings.

Dietary fat and serum lipids

Many studies have demonstrated that the amount and composition of dietary fat are primary determinants of serum cholesterol levels and LDL. It has been concluded that, relative to carbohydrates, the saturated fatty acids elevate serum cholesterol, while the polyunsaturated fatty acids (linoleic acid) lower serum cholesterol, and the monounsaturated fatty acid (oleic acid) has no statistically significant effect (Keys, Anderson and Grande, 1957; Hegsted *et al.*, 1965). While some studies over the past 30 years have yielded various results, on balance the current data (Hegsted *et al.*, 1993) confirm these general conclusions as to the relative effects of saturated, polyunsaturated and monounsaturated fatty acids upon serum cholesterol levels. The specific potency of the saturated and polyunsaturated fatty acids to modify serum lipid levels has not been, and probably cannot be, clearly defined for all conditions. Originally, Keys and his colleagues (1957) thought the combined saturated fatty acids were twice as potent in elevating serum cholesterol as the polyunsaturated fatty acids were in lowering it; later studies led by Hegsted (1965) attributed greater potency to the polyunsaturates. However, individual studies conducted with few subjects and diets may show substantial differences in the effects observed. Combined analyses (Mensink and Katan, 1992; Hegsted *et al.*, 1993) agree that the cholesterol-lowering effect of linoleic acid is 2 to 3 times less than the cholesterol-raising effect of saturates. However, they indicate substantial differences in relative potency depending upon the data selected for analysis. Whether this is due to the traits of individuals, diet characteristics or fats studied, the quality of specific studies, and so forth, is uncertain. It is clear that the amount of dietary fat and its composition are major determinants of serum cholesterol levels; that saturated fatty acids and dietary cholesterol elevate serum cholesterol and that polyunsaturated fatty acids (linoleic acid) have a modest cholesterol-lowering effect relative to carbohydrates.

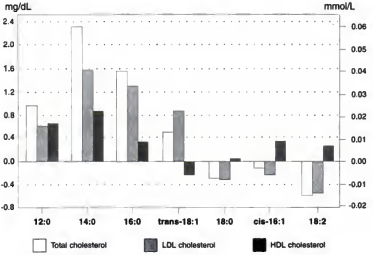
Specific fatty acids and cholesterol

Saturated fatty acids. Early studies (Keys, Anderson and Grande, 1965; Hegsted *et al.*, 1965) suggested that various saturated fatty acids have different effects upon serum cholesterol levels. Particular fats with high levels of stearic acid did not appear to be as hypercholesterolemic as expected from their content of saturated fatty acids. These differences were investigated by the use of transesterified fats, incorporating lauric, myristic, palmitic and stearic acid into olive and safflower oil (McGandy, Hegsted and Myers, 1970). Many of these preparations appeared to be approximately equal in their cholesterol-raising ability, thus suggesting that the position of the fatty acids in the triacylglyceride may be important as well. More recent data (Bonanome and Grundy, 1988) also indicated that stearic acid may not elevate serum cholesterol levels appreciably. Overall, the data indicate that stearic acid in most natural fats has minimal effects upon serum cholesterol levels. It should be specifically noted, however, that the effects of stearic acid and other saturated fatty acids on susceptibility to hypertension, cancer, obesity, and other illnesses, are unknown. Also, data on the activity of saturated fatty acids with regard to thrombotic activity are inadequate. Hence, whether it is desirable to substitute stearate for other fatty acids in the diet is still uncertain.

There is still considerable disagreement about the relative activity of the other saturated fatty acids - lauric, myristic and palmitic. The early data (Hegsted *et al.*, 1965) suggesting that myristic acid was the most hypercholesterolemic of the saturated fatty acids may be due to the designs of the studies. Butterfat and coconut oil, both sources of myristic acid, were the primary saturated fats studied and the intake of total saturated fatty acids correlated with myristic acid intake. A direct comparison of myristic and palmitic acid showed that both raise LDL cholesterol relative to oleic acid, but that myristic acid was slightly more powerful (Zock, 1994). Most other metabolic and epidemiologic studies have not reported the intake of the specific saturated fatty acids (Denke and Grundy, 1992; Doherty and Iacono, 1992; Sundram, Hayes and Siru, 1994). Some animal and human studies (Hayes *et al.*, 1991; Ng *et al.*, 1991) have reported minimal effects of palmitic acid but this may be due to the specific diets utilized (Hayes *et al.*, 1991; Pronczuk, Khosla and Hayes, 1994). In studies of cebus monkeys, it was reported that palmitic acid is only hypercholesterolemic when the cholesterol intake is high (Khosla and Hayes, 1993), however, metabolic studies in normolipidemic volunteers showed that palmitic acid strongly raised total cholesterol (Bonanome, 1988; Denke, 1992; Zock, 1994). Palmitic acid is the major saturated fatty acid in most diets and lauric, myristic and palmitic acid are considered to be the principal hypercholesterolemic fatty acids although they may differ in potency. Recent findings on individual fatty acids are summarized in Figure 9.1.

Polyunsaturated fatty acids. Replacing saturated fatty acids by either oleic or linoleic acid lowers serum cholesterol levels. In many specific studies, it was not possible to determine

FIGURE 9.1
Effects of individual dietary fatty acids on serum total and lipoprotein cholesterol



Note: computed from various sources
Source: Katan, Zock and Mensink, 1993.

whether it was the addition of oleic and/or linoleic acid or the lowering of intake of saturated fatty acids which caused the reduction in serum cholesterol. Predictive equations attribute the changes in serum cholesterol to changes in saturated and polyunsaturated fatty acids while the monounsaturated fatty acid (oleic) was shown to be neutral (Keys, Anderson and Grande, 1957; Hegsted *et al.*, 1965; Mensink and Katan, 1992; Hegsted *et al.*, 1993) or to have a small cholesterol-lowering effect (Mensink and Katan, 1992).

Interestingly, in several studies the linoleic acid content of adipose tissue which is probably a better indicator of usual, long-term, linoleic acid intake than dietary data (van Staveren *et al.*, 1986), as well as the linoleic acid content of serum phospholipids or cholesterol esters, showed an inverse relationship between linoleic acid levels and the incidence of myocardial infarction (Wood *et al.*, 1984; Logan *et al.*, 1978; Valek *et al.*, 1985; Riemersma *et al.*, 1986). This may indicate a protective role of the polyunsaturated fatty acids other than their effect on serum lipid levels (Renaud *et al.*, 1986). Studies with animals (Charnock *et al.*, 1985; Charnock, Abeywardena and McLennon, 1986) indicate that the polyunsaturated fatty acids, especially the n-3 fatty acids, may protect against cardiac arrythmias.

EPA and DHA. In recent years, evidence that fish-consuming populations have a low prevalence of CHD has generated a great deal of interest in fish oils, which are major sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Dyerberg *et al.*, 1978).

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Although the evidence is somewhat controversial, the consumption of such oils appears to have relatively little effect upon LDL and HDL levels but does appear to be effective in lowering serum triacylglycerols and very low-density lipoproteins (Leaf and Weber, 1988). The epidemiological data indicate that there is an apparent protective effect which is probably due to the effects on the thrombotic or immunologic mechanisms rather than effects upon serum lipoproteins. Much of the experimental data has dealt with diets in which fish oils were the major source of dietary fat and it is unlikely that results can be extrapolated to ordinary levels of consumption. This, of course, is not true of the epidemiological findings.

Dietary cholesterol. While it has long been apparent that dietary cholesterol elevated serum cholesterol, the shape of the dose-response curve has been debated. It is clear that the observed responses to changes in cholesterol intake are highly variable and may depend, in part, upon the nature of the dietary fat, cholesterol intake, and perhaps, other dietary constituents (Hopkins, 1992). Hence, as with changes in dietary fat, a good quantitative definition of the response expected from changes in cholesterol intake under all conditions is probably not possible.

It is relevant to note that in the hamster model (Spady and Dietschy, 1985; Woollett *et al.*, 1992) the cholesterol-lowering activity of linoleic acid was clearly evident only when the LDL receptor was adequately suppressed by dietary cholesterol and/or saturated fat. This may explain, in part, the limited potency of linoleic acid which was reported when human subjects were fed formula diets which were low in cholesterol (Hegsted and Nicolosi, 1990).

Intervention trials. Intervention trials generally confirm the effects of dietary fat and cholesterol although the changes observed are often less than in metabolic trials in which better dietary control is possible. For example, a significant drop in serum cholesterol was found when a diet which was low in animal fat and high in polyunsaturated fat was substituted for the usual Finnish diet (Ehnholm *et al.*, 1982) which is high in saturated fat and cholesterol. In southern Italy, the reverse type of study, in which animal fat was substituted for olive oil and carbohydrate, resulted in a substantial rise in serum cholesterol and LDL (Ferro-Luzzi *et al.*, 1984). Relatively small changes in serum lipids have been observed in some field trials (Hunninghake *et al.*, 1993) undoubtedly due to the difficulties in obtaining adequate food consumption data and good dietary compliance in large trials. The Finnish Mental Hospital Study (Turpeinen *et al.*, 1979) reported a significant decrease in CHD in individuals who were fed a diet higher in polyunsaturated fatty acids. When the results of a number of trials, many of which included drugs to lower cholesterol, were summarized (Committee on Diet and Health, 1989; Smith, Song and Sheldon, 1993) the findings were varied but consistent: diet can have a desirable effect in lowering serum cholesterol. Several studies (Blankenhorn *et al.*, 1987; Ornish *et al.*, 1990; Schuler *et al.*, 1992) demonstrated that a severely restricted diet can limit or actually reverse the development of atherosclerotic lesions.

Such immediate effects of dietary modification upon lesion development appear to be consistent with change in the diets of populations, such as those observed in some European countries during World War II (Schettler, 1979). Large decreases in mortality from CHD in some countries have been observed in recent years. They were greater in countries such as the USA and Australia which emphasized dietary modification in the general population (Dwyer and Hetzel, 1980). Overall, the data from field trials support the epidemiologic and metabolic conclusions about the desirability of dietary modifications to lower serum cholesterol levels (Stamler *et al.*, 1993).

Dietary effects on lipoproteins

Low density lipoproteins (LDL) carry most cholesterol and are identified as the most important cause of atherosclerosis. The dietary effects of the various fatty acids and cholesterol upon LDL levels generally parallel those described above for total serum cholesterol (Mensink and Katan, 1992; Hegsted *et al.*, 1993). The saturated fatty acids, presumably lauric, myristic and palmitic acids, elevate LDL levels; linoleic acid lowers LDL levels; and oleic acid appears to be neutral or slightly lowers it relative to carbohydrates. It has recently been suggested that oxidized LDL is the major, perhaps the cause, of atherosclerosis. Oxidized LDL is more readily taken up by the monocytes which leads to the atherosclerotic plaque.

Some studies suggest that various antioxidants limit the development of atherosclerosis in animals and humans (Steinberg *et al.*, 1989; Frei, England and Ames, 1989; Jialal, Vega and Grundy, 1990; Riemersma *et al.*, 1991). Vitamin E, carotenoids and vitamin C have received the most attention but other antioxidants may be effective as well (Bjorkhem *et al.*, 1991). High intakes of vitamin E have been associated with reduced risk of coronary heart disease in both men (Rimm *et al.*, 1993) and women (Stampfer *et al.*, 1993). Hence, the intake and circulating levels of these and perhaps other antioxidants must be considered in assessing the relative risk of disease. Presumably, this will become more important as this field develops. Insufficient evidence is available to indicate the relative importance of the circulating levels of LDL compared to the levels of antioxidants. This does not detract from the convincing evidence that high levels of LDL constitute a major risk of CHD.

Within populations, high levels of high density lipoproteins (HDL) are strongly associated with reduced risk of CHD (Wilson, Abbott and Castelli, 1988; Gordon and Rifkind, 1989; Gordon *et al.*, 1989; Knuiman *et al.*, 1987). HDL is thought to actively protect against CHD (NIH Consensus, 1993) presumably by reverse cholesterol transport, that is, transport from the periphery to the liver although this is uncertain in humans. Within populations, the level of HDL is determined partly by genetic factors and partly by environmental conditions. HDL levels are lowered by smoking, obesity and male hormones, and are raised by physical activity as well as by consumption of alcohol, saturated fats and cholesterol. However, the increase in HDL which is attributable to saturated fats and cholesterol is outweighed by greater increases in LDL. Evidence that manipulation of HDL levels, by dietary or other means, modifies susceptibility to CHD is unavailable and the significance of dietary-induced changes in HDL levels needs to be clarified. Summaries of the current data (Mensink and Katan, 1992; Hegsted *et al.*, 1993) indicate that all three classes of fatty acids tend to elevate HDL levels, with saturated fatty acids being the most potent and linoleic acids being the least influential. On the other hand, low-fat diets which are known to protect against CHD also lower HDL levels (Denke and Breslow, 1988). After HDL is reduced by a low-fat diet, its metabolism is modified (Brinton, Eisenberg and Breslow, 1990). However, there is little reason to believe that dietary modifications which reduce LDL levels are undesirable although they may also reduce HDL levels to some extent.

Trans fatty acids

The effects of *trans* fatty acids have been discussed elsewhere in this report. Available data indicate that the serum lipoprotein responses to monounsaturated *trans* fatty acids approximate the effects of saturated fatty acids. Whether they have specific effects on HDL, as indicated by the studies of Mensink and Katan (1990), or not (Judd *et al.*, 1994) is a question which remains to be clarified.

Conclusion

Abundant evidence supports the conclusion that elevated levels of serum cholesterol and LDL constitute a major risk of atherosclerosis and coronary heart disease. The degree of risk may be modified by various antioxidants and complex interactions between the degree of atherosclerosis, thrombotic and fibrolytic, and vascular reactivity.

When various fats are fed to human beings under controlled conditions, differences in chain length and the number and geometry of the double bonds of the fatty acids induce marked differences in the concentration of lipids and lipoproteins in fasting blood serum. Relative to carbohydrate, the saturated fatty acids - lauric, myristic and palmitic - raise both LDL and HDL cholesterol and lower VLDL cholesterol and triglycerides. In most fats, stearic acid appears to have minor effects. Linoleic acid lowers LDL while oleic acid appears to be neutral. Oleic and linoleic acids may elevate HDL levels modestly relative to carbohydrates with linoleic acid having the least effect. *Trans* isomers of oleic acid elevate LDL levels and may depress HDL levels while the effects on other lipoproteins are, as yet, uncertain. The fatty acids of fish oils lower serum triglycerides markedly but appear to have little effect upon LDL and HDL levels. Dietary cholesterol also elevates LDL levels and probably HDL.

In general, the metabolic studies on the dietary effects of fats and cholesterol upon serum lipids and lipoproteins are consistent with epidemiologic and intervention studies and the trends over time observed in various populations. Each type of study concludes that dietary modifications that lower serum cholesterol and LDL levels decrease the risk of CHD.

Chapter 10

Isomeric fatty acids

Under conditions of partial hydrogenation, a double bond may change from a *cis* to a *trans* configuration (geometric isomerization) or move to other positions in the carbon chain (positional isomerization). Both types of isomerization frequently occur in any one fatty acid undergoing hydrogenation.

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. The most common *trans* fatty acids are monounsaturated, but various diunsaturated *cis*, *trans* and *trans*, *cis* isomers also occur. In *trans* monounsaturated fatty acids of partially hydrogenated oil, the double bond tends to be normally distributed around positions 9 to 11 with a range of 5 to 15 (Dutton, 1979; Marchand, 1982).

Occurrence in diets

The most common sources of isomeric fatty acids are margarines and shortenings containing partially hydrogenated vegetable or fish oil. Dairy products and meat from ruminant animals derive their isomeric fatty acids from the hydrogenation process in the rumen where bacteria carry out anaerobic fermentation. In milk fat, a *trans* double bond may occupy positions 6 to 16, with a preference for position 11. The *trans* fatty acids comprise approximately 5 percent of the total fatty acids in products from cows and sheep, whereas those in commercially hydrogenated fats can range to more than 50 percent (Gurr, 1990).

Whenever partially hydrogenated fats are consumed, both *cis* and *trans* isomers are involved, however, the presence of *trans* fatty acids is used to detect hydrogenated fat. Hydrogenation of polyunsaturated fatty acids enhances the assortment of isomers. Although the levels of *cis*, *trans* and *trans*, *cis* isomers of linoleic acid in stick margarines comprise as much as 7.6 percent of the fatty acids, and fatty acids with two *trans* double bonds as much as 2.8 percent, most are under 1 percent (Ratnayake, Hollywood and O'Grady, 1991). In partially hydrogenated canola oil, the major polyunsaturated isomer was found to be *cis*-9, *trans*-13 octadecadienoic acid (Ratnayake and Pelletier, 1992). The *cis* bond at position 9 prevailed in most isomers of linoleic acid. The occurrence of isomers of polyunsaturated fatty acids can be controlled in the processing of oil. Good oil processing practices provide oils with low levels of *cis/trans* and *trans/trans* isomers.

Estimates of *trans* fatty acid intakes have been controversial. The average intake for the population in the USA, based on disappearance data, was given as 7.6 g/day and later revised to 8.1 g/day (Hunter and Applewhite, 1986; 1991). Other calculations suggested an average intake of 13.3 g/day; this was based on such assumptions as food service and industrial shortenings having 40 percent *trans* fatty acids and salad and cooking oils having 25 percent (Enig *et al.*, 1990, Steinhart and Pfalzgraf 1992, 1994). In West Germany, the intake of *trans*-octadecenoic acid was estimated to be 4.5 to 6.4 g/caput/day with 35-45 percent from ruminant products (Heckers *et al.*, 1979). For the British population, the average intake was given as 7 g/day with a possible range of 5 to 27 g/day, depending on the selection of foods (British Nutrition Foundation, 1987). In India, the average intake of hydrogenated fat having 55 percent *trans* fatty acids was 2.04 g/day (National Council for Applied Economic Research, 1991). An adult living in the Indian states with highest levels of consumption could have an intake of approximately 11 grams or 4 percent of energy per day of *trans* fatty acids. Apparent average levels of intake may not be as relevant to health as higher levels and estimates of the 90th percentile of intake of *trans* fatty acids in each geographical area would be useful.

The consumption of isomeric fatty acids is expected to decrease further as soft margarines and low-fat spreads continue to replace the stick or print-type of product. The replacement of lard or tallow by partially hydrogenated oil, however, would have the opposite effect. Care must be taken when fats are substituted so that a reduction of isomers in one type of food does not lead to an increase in another food.

Metabolic effects in animals

An examination of the early literature showed that many results which originally had been attributed to *trans* fatty acids were in fact confounded by essential fatty acid deficiency (Beare-Rogers,1983; Gurr, 1983). Isomeric monounsaturated acids, like saturated fatty acids, tend to occupy the 1-position and polyunsaturated acids the 2-position of animal phosphoglycerides. Studies of the general pattern of fatty acids in membrane constituents imply that, with adequate polyunsaturated acids, the *trans*-monounsaturated acids do not accumulate in the 2-position of phosphoglycerides nor do they influence eicosanoid production. A level of 2 en% of linoleic acid in the diet was sufficient to prevent an effect on eicosanoid synthesis (Zevenbergen and Haddeman, 1989). When a high dose of *trans*-fatty acids was fed to rats, an assessment of the level of linoleic acid needed to prevent metabolic changes gave the figure of 5 percent of fat or 2 en% (Verschuren and Zevenbergen, 1990). An adequate level of essential fatty acids is, therefore, critical to prevent specific effects of isomeric fatty acids.

***Trans* polyunsaturated fatty acids.** In studies with deuterated isotopes, the *trans*-9, *cis*-12-isomers which accumulated in mouse liver were 2 to 4 times more than the *cis*-9, *trans*-12-isomers (Beyers and Emken, 1991). The action of the *trans*, *cis*-isomer in increasing 9 desaturation was similar to that observed in animals which were deficient in essential fatty acids. The position of the *trans* double bond appears to influence a variety of enzyme activities.

The *trans*-9, *trans*-12-octadecadienoic acid has been extensively studied in animals (Kinsella *et al.*, 1981). This isomer has the greatest potential for interfering at the enzyme level with the metabolism of essential fatty acids and eicosanoids. The production of this isomer is usually kept to a negligible amount and it is expected to remain so.

Human studies

Experimental evidence. The deposition of isomeric fatty acids in human tissue has been of considerable interest. In the United Kingdom, the *trans* fatty acid content of adipose tissue was reported to be 5 percent (Thomas *et al.*, 1981). Analysis of tissues during routine autopsies in the USA showed that the levels of *trans*-octadecenoic acid varied from 0.4 to 5 percent of the total fatty acids, and reflected the distribution of isomers in the dietary fat (Ohlrogge, Emken and Gulley, 1981). The highest level of *trans* isomers occurred in adipose, cardiac and aortic tissues, with triacylglycerol having similar isomeric patterns to the dietary oils, and phosphatidylcholine showing a selective incorporation, *albeit* less dramatic, than that found in animal and *in vitro* experiments (Ohlrogge, Gulley and Emken, 1982). There was a tendency for isomers having a double bond near the terminal methyl group to be retained without an accumulation of any one isomer.

Studies have demonstrated that adult males showed no discrimination in absorption or oxidation of various octadecenoic isomers labelled with deuterium and fed as triacylglycerol (Emken *et al.*, 1979, 1980 a, b; Emken, 1984). After a single meal containing these triacylglycerols, the incorporation of the different isomers was measured in various plasma lipids. The 12-*cis* isomer was incorporated into the 2-position of phosphatidylcholine and could consequently, replace arachidonic acid.

The most prevalent *trans*-polyunsaturated fatty acid in human adipose tissue appeared to be *cis*-9, *trans*-13 octadecadienoic acid (Hudgins, Hirsch and Emken, 1991; Ratnayake and Pelletier, 1992). Its effect on the metabolism of essential fatty acids and eicosanoids is unknown.

Cholesterol. For more than three decades, various results came from attempts to relate the *trans* fatty acids to changes in serum or plasma total cholesterol. These have been reviewed by Hunter (1992) and Wood (1992). Two studies with liquid formula diets published in the same year came to opposite conclusions. When white, Trappist monks and nuns in the Netherlands were provided with a diet containing *trans* monounsaturated fatty acids, serum cholesterol levels were elevated in the presence of dietary cholesterol but not in its absence. Serum cholesterol levels at the end of 4 weeks of experimental feeding were highest in the group receiving the saturated fatty acids (lauric and myristic), followed by the group receiving fat with 38 percent *trans* fatty acids, and then the group receiving oleic acid. Compared to the values at the beginning of the experimental feeding period, cholesterol values of the group receiving oleic acid fell and those of the group receiving the saturates rose slightly (Vergroesen, 1972; Vergroesen and Gottenbos, 1975).

The other major study with liquid formula diets containing cholesterol involved male prison inmates in the USA who were predominantly black and from cities. The dietary fat containing 34 percent *trans* monounsaturated fatty acids did not produce different results than the control fat which was high in oleic acid (Mattson, Hollenbach and Kligman, 1975). A later study of college students indicated that lightly hydrogenated soy oil was less effective than unhydrogenated soy oil in lowering total and low density lipoprotein cholesterol (Laine *et al.*, 1982).

Lipoproteins. The first measurements on lipoproteins in a study of *trans* fatty acids were reported in 1990 (Mensink and Katan, 1990). Normocholesterolemic men and women consumed a diet of mixed foods: one group received a diet high in oleic acid, another had a diet high in *trans* monounsaturated fatty acids, while the third consumed a diet high in saturated fatty acids to provide 10 percent of total energy. The dietary fatty acids were balanced to provide the same amount of stearic acid in each group while the total saturated and polyunsaturated fatty acids (4.6 en%) were similar for the diets high in oleic or *trans* fatty acids. Compared to the oleic diet, the *trans* diet increased LDL-cholesterol and decreased HDL-cholesterol, while the saturated diet increased LDL and produced no change in HDL-cholesterol after 3 weeks. Both the *trans* and the saturated diet increased triacylglycerol. The results on the apoproteins paralleled the LDL and HDL results. The ratio of LDL/HDL was higher for the *trans* diet than for the saturated diet. The data indicated that *trans* fatty acids had no advantage over saturated fatty acids.

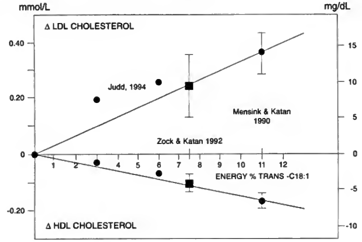
Although the fat used in this work was catalytically isomerized rather than commercially hydrogenated, it did contain the *trans* isomers that are common in "Western" diets. The greatest difference between the test fat and the dietary fat of the USA appeared to be a positional *cis* isomer with a double bond in the 8 position (Mensink and Katan, 1991).

A second experiment from the same laboratory had a lower level of *trans* fatty acids, 7.7 percent, instead of 10 or 11 percent of energy (Zock and Katan, 1992). The *trans* diet was compared to one rich in linoleic acid and one rich in stearic acid, a fatty acid that is not known to raise blood cholesterol levels. Relative to linoleic acid, the isomeric fatty acids increased LDL-cholesterol and decreased HDL-cholesterol.

A study in the USA compared saturated fatty acids, oleic acid and two levels (3.8 and 6.6 en%) of *trans* isomers (Judd *et al.*, 1994). All diets contained at least 10 en% of the saturated fatty acids, lauric, myristic and palmitic acids and 3 en% stearic acid. Linoleic acid was maintained at 6 en% of the diet. The tightly controlled experiment demonstrated differences in the effects of *cis* and *trans* monounsaturated fatty acids, even when they constituted less than 4 en%. After 6 weeks on the test diets, the "moderate trans", "high trans" and saturated diets raised cholesterol levels. In agreement with the results of Mensink and Katan (1990), a diet containing *trans* fatty acids raised LDL-cholesterol and apoprotein B and depressed HDL-cholesterol and to a lesser extent apoprotein A-1, compared to the oleic diet. Statistically significant changes at a lower level of intake were observed since the saturated diet increased both the LDL-cholesterol and the HDL-cholesterol. The ratio of LDL-to HDL-cholesterol was less favourable with the 6.6 en% *trans* diet than with the saturated diet. The diet containing 6.6 en% of *trans* fatty acids, compared to the other diets studied, also elevated blood triacylglycerol. Although the overall changes were small, the *trans* fatty acids and the saturated fatty acids had somewhat similar effects.

The LDL/HDL ratios obtained in the experiments of Mensink and Katan (1990 -Exp.1), Zock and Katan (1992 - Exp. 2), and Judd *et al.* (1994) are indicative of a dose response to *trans*-fatty acids (Figure 10.1).

FIGURE 10.1
Influence of trans fatty acids on LDL and HDL cholesterol levels



A study on mildly hypercholesterolemic males showed that an increase of 4 en% in *trans* monounsaturated acids did not negate the LDL-lowering potential of vegetable oils when the concentration of linoleic acid was approximately 3 times greater than that of palmitic acid (Nestel *et al.*, 1992). Considering most known dietary patterns, such a distribution of fatty acids is highly unlikely.

Other studies involved test fats containing *trans* fatty acids in the diet of free-living normocholesterolemic men (Wood *et al.*, 1993 a, b). A soft, zero-*trans* margarine which was high in linoleic acid significantly reduced total cholesterol, LDL-cholesterol and apolipoprotein B relative to other test diets. There was no change in HDL-cholesterol with the diet of 1:1:2 in saturates, monounsaturates and polyunsaturates. The butter diet elevated total serum cholesterol and LDL-cholesterol, while the hard margarine produced similar results to butter-sunflower oil and butter-olive oil blends. The difference in response to the hard and soft margarines was attributed largely to *trans* fatty acids and supported the findings of Mensink and Katan (1990).

Margarine containing hydrogenated corn oil which was used to replace unhydrogenated corn oil in a diet designed to reduce plasma cholesterol, led to less reduction in total cholesterol, LDL-cholesterol and apoprotein B, but to no difference in HDL-cholesterol nor Lp(a) (Lichtenstein *et al.*, 1993). The diets were already low in saturated fatty acids and cholesterol.

In laboratories in Australia and the Netherlands, lipoprotein (a) [Lp(a)] which is regarded as an independent risk factor for cardiovascular disease, increased in individuals receiving *trans* fatty acids, however, this was not found in the USA. An "elaidic-rich" diet, which might have contained *trans* isomers other than the 9-*trans* isomer, was fed at a *trans* intake of 7 percent of energy as a substitute for oleic acid (Nestel *et al.*, 1992). The resulting LDL levels were similar to those produced by saturated fatty acids and were higher than those obtained with oleic acid. Only the diet containing *trans* isomers led to significant elevations of Lp(a). Similarly, an examination of samples from previous studies on dietary fat and lipoprotein demonstrated that isomeric fatty acids were associated with increased Lp(a) (Mensink *et al.*, 1992). Oleic acid had the effect of decreasing Lp(a) and LDL-cholesterol. It appeared unlikely that dietary effects on LDL-cholesterol and Lp(a) were mediated by the same pathway.

Different protocols were used in the various studies to determine the effect of dietary *trans* fatty acids on plasma lipoproteins. Whether subjects received foods for a complete diet or selected their non-test foods may have influenced the precision attained in an experiment. The part of the diet that was not controlled may have great variations in fatty acid composition, particularly in the content of fatty acid isomers. It may be noteworthy that the studies showing definite changes in both LDL- and HDL-cholesterol had foods prepared for the subjects.

To date, only those *trans* fatty acids found in partially hydrogenated vegetable oils were studied in controlled human investigations. It is not known what effect partially hydrogenated fish oils with C_{20} and C_{22} fatty acids have on human lipoprotein profiles.

Epidemiologic evidence. Epidemiological studies have included prospective, case-control and cross-sectional designs. Persons dying of ischaemic heart disease in the UK had a lower concentration of shorter chain fatty acids and a higher concentration of *trans* fatty acids in adipose tissue fat, and were therefore judged to have consumed less ruminant animal fat and more commercially hydrogenated fat (Thomas and Scott, 1981; Thomas, Winter and Scott, 1983). An attempt to estimate *trans* fatty acid intake in adult men from semi-quantitative food frequency questionnaires indicated that the 10th and 90th percentiles of intake were 2.1 and 4.9 g/day, respectively (Troisi, Willett and Weiss, 1992). These low estimates, compared with the average of 8.1 g/day (Hunter and Applewhite, 1991), showed a weak positive correlation with LDL-cholesterol and an inverse correlation with HDL-cholesterol. In the Nurses' Health Study, the quintiles varied from 1.3 to 3.2 percent of energy or 2.4 to 5.7 g/day and were below the expected average intake (Willett *et al.*, 1993). Such foods as margarine, cookies, biscuits, cake and white bread were used for the calculation of *trans* fatty acids in hydrogenated vegetable fat. The relative risk of fatal and non-fatal myocardial infarction for women in the highest category of *trans* fatty acid intake was 1.6 compared to those with the lowest intakes. In a cross-sectional study of patients undergoing coronary angiography (Siguel and Lerman, 1993), somewhat higher values for plasma *trans* fatty acids were observed in patients than in reference subjects (1.38 percent *versus* 1.11 percent). Other data used to support the hypothesis that the intake of partially hydrogenated vegetable oils may contribute to the risk of cardiovascular disease came from a case-control study where the relative risk for the increasing quintiles of *trans* fatty acid intake were 1, 0.74, 0.43, 0.63 and 1.94 (Ascherio *et al.*, 1994). It is interesting that the third quintile represented less than half the risk of the first quintile and only the highest quintile was associated with increased risk.

One of the great handicaps in such epidemiological studies is the measurement of exposure to isomeric fatty acids. Even within one food group, there is great variation in the concentration of *trans* fatty acids (Ratnayake *et al.*, 1993).

Pregnancy and lactation

Depending on the diet of mothers, human milk may contain various amounts of *trans* fatty acids. Koletzko (1991) detected the transfer of *trans* fatty acids across the human placenta and reported an inverse correlation between *trans* fatty acid exposure and birth weight in premature infants. Further study indicated that the *trans* fatty acids in the plasma lipids of infants were negatively correlated to arachidonic and docosahexaenoic acids (Koletzko, 1992). More studies are required in this area.

If there is a possibility of a deficiency of essential fatty acids during pregnancy and lactation, as observed by Holman, Johnson and Ogburn (1991), the level of intake of *trans* fatty acids could be of concern. On the basis of animal experiments, the intake of linoleic acid is of critical importance when diets contain partially hydrogenated oils.

Conclusions

In controlled metabolic studies using oleic acid as a comparison, *trans* fatty acids from partially hydrogenated vegetable fats raise plasma LDL cholesterol in a manner which is similar to that observed with saturated fatty acids. In contrast to saturated fatty acids, however, *trans* fatty acids do not increase plasma HDL cholesterol and may lower this lipid fraction compared to oleic acid. Thus, the ratio of total cholesterol to HDL appears to be more unfavorable for *trans* fatty acids compared with equivalent amounts of either oleic acid or saturated fatty acids. In two studies, *trans* fatty acids increased levels of lipoprotein (a), another reputed factor for coronary heart disease.

A number of conclusions can be drawn. First, isomeric fatty acids in partially hydrogenated vegetable oils appear to be hypercholesterolemic, however, the interpretation of epidemiological studies is uncertain. Second, where the intake of saturated fatty acids is curtailed, it seems appropriate to curb *trans* fatty acids to improve the profile of plasma lipoproteins. Consumers should be encouraged to substitute liquid oils, soft margarines and spreads for hard fats, wherever feasible. Third, where there is a possibility of a deficiency of essential fatty acids during pregnancy and lactation, a high level of intake of *trans* fatty acids should be avoided. Fourth, food processors are encouraged to reduce the isomerization of fatty acids as much as possible. It is unacceptable to use marketing claims such as "low in saturates" when the product is high in *trans* (unsaturated) isomers. Fifth, governments should monitor the intake of fatty acid isomers and regulate the claims being made with respect to products containing them.

Chapter 11

Cancer and dietary fat

Early studies clearly demonstrated that mice and rats which were fed high-fat diets were far more susceptible to skin and mammary cancer than animals fed diets low in fat (Tannenbaum and Silverstone, 1953; Tannenbaum, 1959). These studies attracted little attention until epidemiologic data demonstrated that the prevalence of cancer at several sites was much higher in countries with high-fat diets than in those with low-fat diets (Carroll, 1975; Carroll and Khor, 1975). Since that time, abundant experimental data have shown that feeding rats or mice a variety of high-fat diets increases susceptibility to cancer of the breast, skin, colon, pancreas and prostate. Such studies have used a variety of carcinogens to induce cancer or, in some cases, spontaneous tumours. Much of the evidence relating diet to cancer has been presented and discussed in recent reviews (U.S. Department of Health and Human Services, 1988; National Research Council, 1982, 1989).

Epidemiology

Several types of epidemiologic data have been reported. The first can be categorized as inter- and intra-country correlations.

Data collected by WHO on cancer incidence or mortality have been adjusted for age and standardized (Kurihara and Aoki, 1984; Parken *et al.*, 1992) and then compared to dietary fat disappearance data collected by FAO (FAO, 1980b). Fat disappearance data do not take wastage into account and the early data, expressed as grams consumed per person per day, grossly overestimated actual intake. However, when fat is expressed as a percentage of total energy, the values approximate usual consumption and show a strong positive correlation with cancer mortality.

Because the populations compared are large in size, variations in dietary habits and genetic susceptibility are taken into account in these studies. Therefore, it is likely that the observed differences can be safely attributed to environmental factors. On the other hand, the accuracy of mortality data differs for each country and type of cancer. Similarly, the reliability of the fat disappearance data varies and the crude estimates of fat intake give no indication of the dietary practices of those individuals who do or do not develop cancer.

Comparisons of groups within countries, such as studies in the USA comparing the Seventh Day Adventists with the population as a whole (Phillips *et al.*, 1980) and ethnic groups in Hawaii (Kolonel *et al.*, 1982) have been presented. These involve smaller groups than the inter-country comparisons and the correlations with fat intake are generally weaker.

Time trends and migrant studies provide a second type of epidemiological data. Changing patterns of diet and cancer within countries or in groups who have migrated provide some of the strongest evidence that environmental factors, rather than heredity, are responsible for geographic differences in cancer incidence and mortality. Such trends in Japan (Hirayama, 1979) and among migrants from Japan (Prentice and Sheppard, 1990), China and various European countries (Gori, 1978; McMichael and Giles, 1988; Prentice and Sheppard, 1990) generally indicate that the trend from low-fat to high-fat diets is accompanied by increases in cancer at sites, such as breast and colon, that are positively correlated with fat intakes in the intercountry data. There are no reports on migrants changing from high- to low-fat diets; if such data exist, it would be of considerable interest.

Case-control studies give a third type of evidence. The diets of groups of people with cancer have been compared with those of individuals of similar age without cancer. Generally, these studies have failed to indicate a connection between diet and cancer. Such comparisons involve individuals who are clearly susceptible to cancer or those who have been exposed to adequate initiating stimuli; these factors may not be true of the people in the control group. Such studies depend upon the ability of individuals to accurately recall their dietary habits over the years which is probably unrealistic. Also, their current dietary habits may be influenced by the presence of disease. Substantial data indicate that dietary fat may act primarily during the promotional stage of carcinogenesis. Hence, it is not surprising that these kinds of data do not provide strong evidence of an association between diet and cancer (Carroll, 1994).

A fourth type of epidemiological data comes from cohort studies. In these studies, a group of people who are free of disease are followed over time after their diet and other characteristics have been assessed. As some individuals develop cancer or other diseases, attempts are made to compare the characteristics of those who have developed disease with those who have not. Such studies are advantageous in comparison to case-control studies since there is no selection bias with regard to susceptibility to disease. Furthermore, the current diet and other characteristics can presumably be evaluated more accurately than past characteristics. However, the group usually has rather homogeneous dietary practices and it is uncertain that the techniques available can correctly define the eating habits of the individuals. Correlations between nutrient intake assessed by different methods are relatively poor (Willett *et al.*, 1985) and the limitations of this approach have frequently been noted (Goodwin and Boyd, 1987; Freudenheim and Marshall, 1988; Hebert and Kabat, 1991; Hegsted, 1989).

Intervention studies

In clinical trials, the participants are randomly assigned to a "treatment" and a "control" group; the "treatment" group is given counselling on the appropriate diet or other treatment and the "control" group is given standard advice. The groups are monitored over time to determine compliance and who develops disease. Many consider this approach to be the proper way to establish proof of the effectiveness of diet or other methods in treating or preventing disease. The problems in dietary intervention trials, however, are formidable. The groups must be large and the studies are labour-intensive, expensive and must extend over long periods of time. A primary problem is the assessment of individual dietary practices within the groups, particularly when in many instances, the general public is receiving the same advice as the treatment group. Since relatively few individuals can be expected to develop disease, the diets of individuals must be identified to ascertain the degree of compliance in those who do or do not develop disease. Current methods of assessing individual intakes show considerable bias in reported energy intakes, apparently regardless of the methodology used (Livingstone *et al.*, 1990; Schoeller, 1990; Black *et al.*, 1993). It is apparent that participants in treatment groups are under considerable pressure to report consumption patterns which approximate the instructions. In large-scale attempts to test low-fat diets, study participants have reported energy intakes that are unreasonably low, thus casting doubt on the reliability of the food consumption data (Henderson *et al.*, 1990; Hunninghake *et al.*, 1993). Such data appear to confirm the conclusion that the more dietary advice groups receive, the less reliable the data on food consumption become (Mertz, 1992). Thus, the utility or practicality of intervention trials to test dietary effects on disease incidence is questionable.

Experimental studies with animals allow absolute dietary control, can be continued over the lifetime of the animal and have been used to test a wide range of carcinogens and types of diet. The question is whether results found for animals can be extrapolated to humans. If the effects of dietary constituents are consistent with the epidemiologic correlations which have been observed, it is plausible that there are causal relationships between diet and cancer.

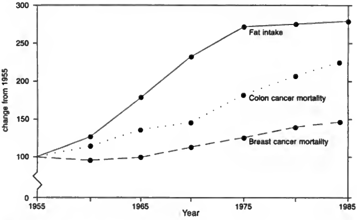
Dietary fat and cancer of the breast and colon

Epidemiological evidence. Cancer of the breast and colon account for a large proportion of the total cancer in Western populations. Both show a strong positive correlation with fat consumption in inter-country studies. Time trends and migrant studies are consistent with these patterns. However, in Japan, colon cancer has increased more rapidly than breast cancer as fat intake has risen (Figure 11.1). Migrants also show a more rapid increase in colon cancer (Berg, 1975) than breast cancer.

In case-control studies, dietary fat appears to be more consistently associated with colon cancer than breast cancer (Carroll, 1994; Willett, 1989), which would be expected because of the longer latent period for breast cancer. Although some studies show a positive association of breast cancer with dietary fat and others do not, a combined analysis of 12 case-controlled studies indicated a statistically significant, positive association with saturated fat intake and breast cancer in post-menopausal women (Howe, 1990). Case-control studies have generally shown a positive relationship between fat intake and colorectal cancer (Whittemore *et al.*, 1990).

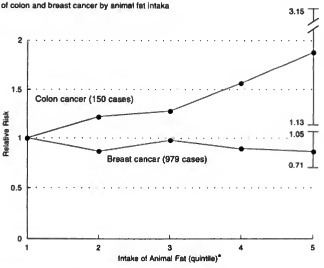
Cohort studies have usually failed to show an association between dietary fat and breast cancer (Howe *et al.*, 1991; Van den Brandt *et al.*, 1993; Willett *et al.*, 1987, 1992). In their study of American nurses, Willett and his colleagues (1990) reported that colon cancer was positively associated with dietary animal fat, saturated fat and monounsaturated fat but the associations were weak and non-significant when adjusted for total energy intake (Figure

FIGURE 11.1
Relative changes in fat intake and age adjusted mortality for breast and colon cancer in Japan



Note: From 1955 to 1985 total energy intake remained nearly constant
Source: Reproduced from Willett, 1989

FIGURE 11.2
Relative risk of colon and breast cancer by animal fat intake



* adjusted for total energy intake
Note: The bars represent 95 percent confidence intervals for the women in the highest quintile
Source: Willett, et al., 1990

11.2). Studies of this kind involve estimates of current diets and do not preclude the possibility that a life-long exposure to a low-fat diet might reduce susceptibility to breast and colon cancer.

Animal experiments

Studies with experimental animals have shown with remarkable consistency that a high-fat diet increases the incidence and yield of mammary and colon cancer (National Research Council, 1982, 1989; Welsch, 1992). The effect appears to be primarily at the promotional stage of carcinogenesis (Carroll, 1975) although initiation may be influenced to some degree (Rogers and Lee, 1986).

Animal studies also indicate that non-lipid materials influence cancer incidence. Rats which were fed commercial chow had fewer tumours than those fed purified diets of similar lipid content (Carroll, 1975; Ip, 1987). There is accumulating evidence that diets which are high in dietary fibre, fruits and vegetables reduce cancer risk in humans (National Research Council, 1982, 1989; Ziegler, 1989; Block, Patterson and Subar, 1992; Sandler *et al.*, 1993; Hunter *et al.*, 1993). In animal experiments, a variety of anticancer agents have been identified in natural materials (Birt and Bresnick, 1991; Wattenberg, 1992). A reduction in fat intake often results in increased consumption of non-fat constituents in the diet. Hence, the difference in cancer incidence and mortality in populations consuming high- and low-fat diets may involve a variety of dietary components other than fat. However, studies to observe the effects of dietary fat using purified diets clearly implicate fat.

Experimental studies demonstrate that there is a requirement for n-6 polyunsaturated fat in the case of mammary tumours. Tumour yields increase with additions of linoleic acid up to a threshold of about 4-5 percent of total calories. When this threshold is reached, increasing total fat causes further increases in incidence and yield, apparently independent of the type of fat added (Ip, 1987). Colon cancer also seems to have a requirement for linoleic acid although this appears to be smaller than that required for mammary cancer (Bull, Bronstein and Nigro, 1989). There have been few studies on the effects, if any, of other specific fatty acids.

Fish oils, containing mainly n-3 polyunsaturated fatty acids, do not appear to promote mammary cancer when fed at high levels although they may have a stimulating effect at low levels (Carroll, 1989; Cave, 1991a,b). Studies with mixtures of n-3 and n-6 fatty acids indicate that the promotional effect of n-6 fatty acids may be neutralized by a high ratio of n-3 to n-6 fatty acids in both mammary and colon cancer (Cave, 1991a; Reddy, 1992).

There has been much speculation about the mechanisms involved in the promotion of mammary cancer by dietary fat. This has included effects on the endocrine and immune systems; changes in the amount or composition of adipose tissue; changes in the fatty acid composition of the membranes of cancer cells; and effects on eicosanoids and lipid peroxidation (Welsch, 1987, 1992).

Many have suggested that energy balance is more important than the level of dietary fat (Boutwell, 1992; Pariza, 1988; Welsch, 1992) since the stimulating effect of high-fat diets can be overcome by caloric restriction. The degree of caloric restriction imposed in most experimental studies, however, is unrealistic for humans. Obesity is often associated with increased risk of breast cancer (Dao and Hilf, 1992), especially when the fat is more localized in the upper body (Ballard-Barbash *et al.*, 1990; Schapira *et al.*, 1990). Lean athletes have been reported to be at reduced risk of breast cancer (Frisch *et al.*,1992). It has been suggested that this may be related to the ability of adipose tissue to convert androgens to estrogens. Exercise in animals is reported to either inhibit or enhance mammary cancer depending upon the intensity and duration of the exercise (Thompson, 1992).

The promotion of colon cancer by dietary fat could be due to enhanced excretion of bile acids and might be mediated by protein kinase C and/or ornithine decarboxylase. The lack of promotion of colon cancer by fish oils may involve effects on eicosanoid production. Prostaglandin inhibitors have been shown to inhibit colon carcinogenesis (Reddy, 1992).

Cancer at other sites

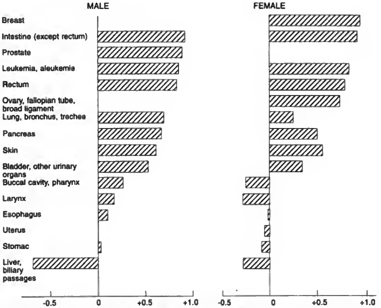
There have been a limited number of investigations of the relationship between dietary fat and cancer at the following sites: pancreas, prostate, skin, lung, ovary, bladder, oral cavity, non-Hodgkin's lymphoma and leukaemia. The inter-country correlations show prostate cancer to be strongly correlated with fat while the relationship is weaker for other sites. In Figure 11.3, the correlation coefficients between the availability of dietary fat and the age-adjusted mortality rates for malignant neoplasms in different countries are illustrated.

In most case-control studies, prostate cancer has been positively associated with dietary fat (Carroll 1994; Rose and Connolly, 1991). The results have been quite variable with regard to other sites but the data available are very limited. A few cohort studies have included pancreatic cancer (Mills *et al.*, 1988) and prostatic cancer (Mills *et al.*, 1989; Severson *et al.*, 1989) and have shown no association with dietary fat. Bosland (1988), however, concluded that a high-fat diet is involved in the etiopathogenesis of prostate cancer.

Experimental studies. Animal models are not available for the study of many cancers. The effects of dietary fat have been studied in rats treated with azaserine and hamsters treated with N-nitrosobis(2-oxypropyl)-nitrosamine. High levels of dietary fat enhance the tumours in the promotional stage (Roebuck, 1992). In the rat model, there appeared to be a rather high requirement for linoleic acid, 4-8 percent in a 20 percent total fat diet. High levels of fish oil reduced the lesions; when mixtures of n-3/n-6 oils were fed, the threshold appeared to be between 3-6 percent linoleic acid. Caloric restriction also reduced focal neoplastic lesions (Roebuck, 1992; Roebuck, Baumgartner and Mac Millan, 1993). Some progress is being made in the development of an animal model for the study of prostate cancer and this provides evidence that high fat diets enhance risk at this site (Pollard and Luckert, 1986).

Many of the earliest studies with animals involved skin cancer and showed a positive association with dietary fat. In one model it was reported that, contrary to the results with breast cancer, there was an inverse correlation between linoleic acid and skin cancer (Fischer *et al.*, 1992). Skin cancer can also be inhibited by caloric restriction (Birt *et al.*, 1993).

FIGURE 11.3
Dietary fat in relation to cancer at specific sites



Correlation coefficients between dietary fat available for consumption in different countries and age-adjusted mortality in those countries from malignant neoplasms at different sites.
Source: Reproduced from Carroll et al., 1986

Conclusion

There are abundant data showing that animals fed high-fat diets *ad libitum* develop tumours of the mammary gland, intestine, skin and pancreas more readily than animals fed low-fat diets, although this effect can be overridden by caloric restriction. These data are consistent with the inter-country correlations linking dietary fat with cancer of the breast, colon, pancreas and prostate. Cohort and case-control studies cast some doubt on these associations, but there are serious limitations in case-control and cohort studies.

In animal experiments, the type of dietary fat, particularly the level and type of the polyunsaturated fatty acids, does have an effect. However, often the animal studies have been conducted with a single source of fat, a situation not found in human populations. The inter-country studies indicate that, over a range of n-6 fatty acids of about 4 to 8 percent of energy, there appears to be no correlation with breast cancer (Carroll *et al.*,1986). Whether such animal data are relevant to humans may be questionable.

Less data are available on the n-3 levels of fatty acids in human diets and their relevance to cancer in humans. However, the total fat content of the diet, rather than the type of fat, appears to have more influence on cancer in human populations.

The association of some tumours with obesity lends some credence to the possibility that the effects of high-fat diets on cancer may be partially explained by changes in energy balance. Lean athletes appear to have less breast cancer. Low-fat diets are reported to be associated with decreased energy intake and weight reduction (Boyd *et al.*, 1988; Henderson *et al.*, 1990) whereas most efforts to control obesity by simple caloric restriction fail. In addition, low-fat diets are inherently higher in crude carbohydrate sources, dietary fibre and fruits and vegetables which appear to provide additional protection against cancer. Appropriate physical exercise is relevant to cancer prevention as it is part of a generally healthy life-style.

Some concern has been expressed about the possibility that low serum cholesterol levels may be associated with increased risk of cancer, particularly cancer of the colon (McMichael, 1991). However, since colon cancer is rare in populations consuming low-fat diets and having low serum cholesterol levels, it is evident that a low serum cholesterol *per se* does not increase risk of cancer.

Chapter 12

Dietary fat and immune response

Interest in the contribution of lipids to immune function began with the recognition of their modifying effect on the reticuloendothelial system (Di Luzio, 1972). An additional stimulus for this interest was the suggestion that an association may exist between certain types of cancer and the quality and quantity of dietary lipids (Carroll and Khor, 1975; Tannenbaum and Silverstone, 1953). Also, dietary fat has been shown to affect the severity of autoimmune diseases as well as the length of allograft acceptance (Mertin and Hunt, 1976; Ring *et al.*, 1974). Furthermore, the *in vitro* addition of fatty acids to lymphocyte cultures changed their mitogenic response (Mertin and Hughs, 1975).

Cooperation between different cells of the immune system via membrane-associated events and through different protein and lipid mediators is essential in mounting a successful immune system response. Dietary lipids can affect the immune system by influencing substrate availability in the formation of cyclooxygenase and lipooxygenase products. These products, in turn, act as lipid mediators in the control of the immune system (Rola-Plaszczynski, 1985 and Goodwin, Messner and Peake, 1974). Furthermore, the cells of the immune system are highly dependent on cell membrane function for operations such as the secretion of lymphokines and antibodies, antigen reception, lymphocyte transformation, and contact lysis. The importance of lipids in the maintenance of membrane integrity (Stubbs and Smith, 1984) indicates that they are potentially critical nutrients in the regulation of the immune function.

Arachidonic acid (AA) metabolites

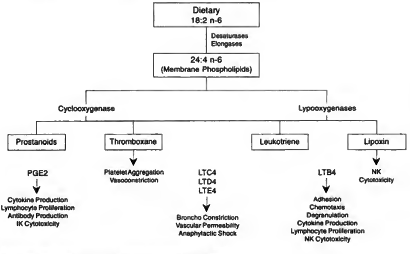
Arachidonic acid (AA) metabolites play a role in control of the immune system. Arachidonic acid (AA) metabolites, prostaglandins (PG), hydroxyeicosatetraenoic acid (HETE) and leukotrienes (LT), are produced by human peripheral blood mononuclear cells (PBMC) and by mouse splenocytes in response to stimulation by mitogens or antigens. They also inhibit subsequent T cell proliferation. Similarly, inhibition of PG synthesis *in vitro* enhances T cell proliferation. Cellular and humoral immune responses operate under negative control by PG. *In vitro*, PGE_2 inhibits T cell proliferation (Goodwin, Messner and Peake, 1974; Webb, Rogers and Nowowiejiski, 1980; Rola-Plaszczynski and Lemaire, 1985; Metzger, Hoffeld and Oppenheim, 1980; Fisher and Bostic-Bruton, 1982; Muscoplat, Rakich and Thoen, 1978), lymphokine production (Gordon, Bray, and Morley, 1976), the generation of cytotoxic cells (Plaut, 1979), and natural killer cell (NK) activity (Roder and Klein, 1979).

In addition to PGE_2, lipooxygenase products, that is, LT and HETE, have been shown to inhibit lymphocyte proliferation in mouse splenocytes and human peripheral lymphocytes (Goodman and Weigle, 1980). This effect may be mediated by decreased T helper and increased T suppressor/cytoxic cell proliferation (Payan, Missirian-Bastian and Goetzl, 1984; Gualde *et al.*, 1984). In some but not all studies, LTB_4 has been shown to increase interleukin (IL)-1 as well as IL-2 production and lymphocyte proliferation (Rola-Plaszczynski and Lemaire, 1985). Lipoxins (Lx) which are products of 15-lipoxygenase of AA have been shown to inhibit human NK activity when tested against K562 target cells (Ramstedt *et al.*, 1985). On the other hand, LTB_4 was shown to augment human NK activity (Rola-Plaszczynski, Gagnon and Sirois, 1983). The effect of AA metabolites on cell-mediated immunity is summarized in Figure 12.1.

Polyunsaturated fatty acids (PUFA)

Several investigators have evaluated the effect of dietary fat on the immune response (Johnson and Marshall, 1984; Erickson, 1986). Review of the literature presents a confusing picture mainly because the effect of PUFA on the immune response varies depending on a number of factors such as: concentration of dietary fat; duration of supplementation; genetic variation; existence of infectious or autoimmune and inflammatory diseases; age of the animal; status of other nutrients such as vitamin E, where the requirement is affected by the degree of saturation of fatty acids; presence or absence of essential fatty acid deficiency in the control diet; particular immunological test used in the experiment; and, series of PUFA tested that is, n-6 or n-3 PUFA.

FIGURE 12.1
Role of eicosanoids in regulation of immunological and inflammatory processes



Source: Reproduced with permission of *Nutrition Reviews* and S.N. Meydani

Plant-derived (n-6) PUFA. The effect of n-6 PUFA of plant origin on the immune response was recently reviewed (Meydani *et al.*, 1991b; Dupont *et al.*, 1990). In the majority of animal studies, essential fatty acids (EFA) were necessary for normal immune function. Essential fatty acid deficiency impairs B and T cell-mediated responses. These impairments are normalized by the inclusion of essential fatty acids in the diet. High-fat diets (about 45 en%), regardless of the degree of saturation, suppress *in vivo* and *in vitro* indices of the immune system. With few exceptions, when animals which were not deficient in fatty acids were fed PUFA of plant origin (high percentage of n-6 FA) they had similar responses to those fed the same level of saturated fatty acids from animals or plant origin. This is especially evident at moderate levels of dietary fat (around 22 en%). At higher levels of dietary fat (around 45 en%) or when mice were challenged with infectious agents or chemical carcinogens, PUFA-fed mice tended to have lower responses to some, but not all, of the immunological tests than those mice fed the same level of saturated fatty acids. These conclusions cannot be readily extrapolated to humans because the immune systems of different species and strains within species respond differently to dietary fat alterations. Well-controlled human trials are needed to determine the effects of dietary fat levels and saturation on human immune responses.

The few studies of the effect of dietary fat on immune response in humans have been epidemiological, retrospective studies. Double-blind prospective studies of multiple sclerosis (MS), a possibly autoimmune demyelinating disease with lipid changes (Bornstein, 1973), suggests a deficiency of PUFA (Bernsohn and Stephanides, 1967; Mertin and Meade, 1977). One study showed that MS patients have less than normal linoleic acid in their lymphocyte lipids and that MS patients supplemented with safflower oil for two years had less frequent and less severe relapses than those supplemented with olive oil (Millar *et al.*, 1973). Another study showed that lymphocytes from MS patients had lower mitogenic responses than control subjects and that supplementation with evening primrose seed oil (which contains 8 percent oleic, 75 percent linoleic, and 9 percent γ-linoleic acid C18:3n-6) for 85 days improved the mitogenic response (Offner, Konat and Clausen, 1974).

Supplementation of MS patients and healthy subjects with 21 g/day safflower oil for 5 weeks increased agglutination with both measles antigen and concanavalin A (Con A), but supplementation with olive oil had no effect (Utermohlen *et al.*, 1981). PUFA supplementation, combined with conventional immunosuppressive therapy, facilitated the acceptance of kidney grafts in humans (Uldall, 1974).

In a cross-sectional study of lymphocyte mitogenesis to B and T cell mitogens and NK activity in 94 free-living subjects, there was no correlation between the degree of adipose tissue fatty acid unsaturation (presumably reflecting dietary consumption) or plasma lipoprotein and the immunological indices (Berry *et al.*, 1987). The inter-individual variation and the influence of other dietary factors could mask a possible effect of fat.

A recent study showed that long term consumption of low fat diets with a moderate level of (n-6) PUFA (6 percent) had no effect on delayed-type hypersensitivity skin response (DTH) which can *in vivo* measure T cell-mediated functions in healthy subjects. It did, however, significantly increase the proliferative response of lymphocytes to T cell mitogen Con A and *ex vivo* production of IL-1β.

Plant-derived (n-3) PUFA. Alpha-linolenic (C18:3n-3) can be converted to EPA (C20:5n-3) which can replace arachidonic acid in membrane phospholipids and be preferentially used by cyclooxygenase. This results in reduced production of PG of the 2 series and leukotrienes of the four series. Thus, it is proposed that consumption of oils containing C18:3n-3 might be associated with immunological changes. This proposition is supported by the many studies which have shown immunological modulation following marine oil consumption. There are very few immunologic studies of plant oils containing (n-3) PUFA. Plant oils containing (n-3) PUFA include perilla seed oil, which has the highest content of (n-3) PUFA (64 percent), followed by linseed oil (62 percent), canola oil (10 percent), and soybean oil (7 percent). In extrapolating the results obtained with (n-3) PUFA of marine oil to oils containing C18:3n-3, two questions should be kept in mind: first, what is the conversion rate of C18:3n-3 to longer-chain (n-3) PUFA (that is, C20:5n-3 and C22:6n-3); and second, is the percentage of (n-3) PUFA in the oil adequate to modify arachidonic acid metabolism and thereby immunological and inflammatory processes?

A recent review of the literature (Meydani *et al.*, 1991b) indicates that consumption of oils containing C18:3n-3 is associated with a modest decrease in eicosanoid production and has no or a slight stimulatory effect on immune response. Long term consumption of low fat diets containing 1.8 percent 18:3n-3 resulted in a significant decrease in plasma AA level associated with a significant increase in T cell mitogenic response to Con A and IL-1β production (Meydani *et al.*, 1993) in humans. Other immunological indices were not affected.

Marine-developed (n-3) PUFA. Different aspects of inflammatory and cell-mediated immune response are under control by PGE_2. PGE_2 production is decreased following consumption of (n-3) PUFA. Several investigators have studied the effect of increased intake of these fatty acids on cytokine production and cell-mediated immune responses. Human studies have consistently demonstrated a decrease in production of pro-inflammatory cytokines when moderate to high levels of marine-derived (n-3) PUFA are taken orally (Meydani *et al.*, 1993; Kremer *et al.*, 1987; Meydani *et al.*, 1991a; Endres *et al.*, 1989). The decrease in production of pro-inflammatory cytokines and eicosanoids contributes to the beneficial effect of fish oil in reducing pathogenesis of inflammatory and atherosclerotic diseases.

Early epidemiological studies demonstrated low incidence of atherosclerotic, inflammatory and autoimmune diseases in Greenland Eskimos who consumed diets high in marine-derived (n-3) PUFA. A modest beneficial effect of fish oil has been demonstrated in human studies of arthritis (Kremer *et al.*, 1990). Fish oil supplementation reduced the clinical symptoms of psoriasis, a skin inflammatory disease (Ziboh *et al.*, 1986). A modest beneficial effect of fish oil was also demonstrated in ulcerative colitis (Lorenz *et al.*, 1989).

Several studies have evaluated the effect of fish oil supplementation on cell-mediated immunity. Also, *in vivo* supplementation with fish oil has been shown to decrease T- and B-cell proliferation and DTH, an *in vivo* measure of cell-mediated immunity (Meydani *et al.*, 1991; Kramer *et al.*, 1991; Santoli and Zurier 1989; Virella *et al.*, 1989).

The immunosuppressive effect of fish oil was also observed in subjects (more than 40 years old) consuming diets based on the National Cholesterol Education Panel (NCEP) recommendation (NCEP Step-2) enriched with fish-derived (n-3) PUFA (providing 0.54 percent energy or 1.23 g/day EPA and DHA) for 6 months (Meydani *et al.*, 1993). This

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long-term consumption of the NCEP Step-2 diet enriched with fish caused a small but significant decrease in the percentage of helper T cells, whereas the percentage of suppressor T cells was increased. This change was accompanied by a significant reduction in DTH and mitogenic response to concanavalin A. A significant correlation between changes in DTH and plasma EPA levels was observed. As reported with fish oil supplementation, the production of cytokines, IL-1β, TNF-α, and IL-6 by PBMCs was also reduced (*Ibid.*). In contrast, long-term feeding of the NCEP Step-2 diet low in fish (0.13 percent energy or 0.27 g/day from EPA and DHA but with the same level of total PUFAs, that is, 10 percent) increased PBMC mitogenic response to the T-cell mitogen concanavalin A and increased IL-1β and TNF-α production but had no effect on the production of IL-6 or DTH. Although the NCEP Step-2 diet enriched with fish-derived (n-3) PUFA caused a significant decrease in the plasma ratio of tocopherol to PUFA the NCEP Step-2 diet low in fish did not cause a significant decrease in this ratio. The results of this study, as well as those observed in animal studies described above, indicate that the level of (n-3) PUFA and tocopherol status are important determinants of the nature of the effect on the immune response which was observed after consumption of these fatty acids.

The reduced production of pro-inflammatory cytokines and pro-aggregatory eicosanoids might be beneficial in cases where excess levels of these mediators are produced, for example, in the case of inflammatory diseases. However, in healthy subjects or those with compromised immune status, reduction in cytokines or eicosanoids production could compromise their normal biological functions which might result in an impairment of host defense or normal platelet aggregation. This can be better understood when the dual function of these compounds, that is, the physiological regulatory role as well as the contribution to the pathogenesis of diseases is considered.

Along these lines, fish oil supplementation has been shown to decrease IL-2 production, T cell mitogen-induced proliferation and the DTH in animal and human models. Increased cerebral hemorrhage has also been observed with fish oil consumption. Furthermore, fish oil feeding inhibited the ability of mouse accessory cells to present antigen to T helper cells (Fujikawa *et al.*, 1992) and mice which were fed fish oil had a reduced capacity for cytotoxicity of mastocytoma cells upon stimulation with LPS or INF-γ (Hubbard, Somers and Erickson, 1991).

It has been shown that long-term consumption of moderate levels of marine-derived (n-3) PUFA decreases percent T helper cells, mitogenic response to T cell mitogen (by 34 percent) and DTH (by 50 percent) (Meydani *et al.*, 1993). The decrease in DTH is of particular interest as this test has been shown to be a predictor of morbidity and mortality. The clinical significance of this finding is supported by the observations that fish oil feeding decreased natural resistance of mice to infection with *Salmonella typhimurium* (Chang *et al.*, 1992), and rats fed diets containing 9 percent menhaden oil had a shorter life span compared to those fed corn oil or beef tallow (Berdanier *et al.*, 1992). Rabbits given a high fish or safflower supplement (5 g/kg.d) for 7 days after birth had decreased lung clearance of inspired *Staphylococcus aureus* by ~50 percent compared with saline control or low doses of the oils (0.22 g/dg.d) (D'Ambola *et al.*, 1991). Fish oil diets also augmented the non-histamine-mediated bronchoconstrictor response in pulmonary anaphylaxis (Lee *et al.*, 1985). Further studies are needed to determine the effect of fish oil supplementation on host defense in humans.

Without adequate antioxidant protection, the substitution of membrane fatty acids with highly oxidizable (n-3) PUFA of fish oil, that is, EPA and DHA, may potentiate the peroxidation of cellular membranes. Increased free radical activity has been demonstrated in laboratory animals fed diets high in (n-3) PUFA (Nalbone *et al.*, 1989; Herbert and Wills 1987; Hu M-L *et al.*, 1989). These compounds might contribute to the suppressive effect of fish oil on T cell-mediated functions. These risks which are associated with intake of (n-3) PUFA can be minimized by the intake of appropriate levels of an antioxidant nutrient such as vitamin E without compromising its beneficial effects.

Conclusion

Studies with animals, tissue cultures, as well as humans indicate that both the level and degree of saturation of dietary lipids influence inflammatory and immunological responses. The nature of the effect is dependent on the type of fatty acid, age, antioxidant and health status of the subject. Some of these immunological changes are associated with beneficial clinical effects, for example, reduction in pro-inflammatory cytokine by marine-derived (n-3) PUFA. Others might not be desirable, for example, decreased T cell-mediated function by marine-derived (n-3) PUFA in older people.

Thus, the effect of lipids on immune response should be considered before recommendations to change dietary lipids for other health benefits are made. Furthermore, recommendations to increase PUFA intake should be accompanied by appropriate recommendations for an increase in antioxidant intake, in particular vitamin E.

In general, for healthy individuals, recommendations to decrease dietary total fat intake, with the inclusion of moderate amounts of (n-6) and (n-3) PUFA with adequate antioxidant nutrients should provide for a competent immune response.

Chapter 13

Dietary fat, hypertension and stroke

While salt intake and obesity have been related to hypertension, studies on the possible roles of dietary fat in the regulation of blood pressure and the pathogenesis of hypertension have shown many inconsistent results (Beilin, 1987; Sacks, 1989; Iacono and Dougherty, 1993).

Cholesterol and total fat

In stroke-prone, spontaneously hypertensive rats, cholesterol-rich, high-fat diets decreased blood pressure and decreased the incidence of stroke (Yamori, 1977). This may be due to the attenuation of vascular activity (Yamori, 1981). Epidemiological data are generally consistent with the animal experiments, they indicate that diets which are very low in fat increase the occurrence of some forms of stroke (Jacobs *et al.*, 1992). Societies with a low intake of fat and animal protein, such as traditional Japan, tend to have high rates of haemorrhagic stroke. An elevated risk of stroke is found among segments of the Japanese population with low levels of serum cholesterol, particularly among those with high blood pressure (Komachi *et al.*, 1976). In a large, screened population of men in the USA, those with the lowest serum cholesterol levels had an elevated risk of haemorrhagic stroke, even though the risks of ischemic stroke, coronary heart disease and total cardiovascular disease were positively and linearly related to serum cholesterol (Kagan, Popper and Rhoads, 1980; Iso *et al.*, 1989). While not proving causality, the rates of stroke have declined greatly in Japan since the early 1950s, during which time the amount of fat consumed increased from about 10 percent to 25 percent of total dietary energy. This increase was due primarily to the greater consumption of animal fat.

A number of large epidemiological studies (Kay, Sabry and Csima, 1980; Salonen, Tuomilehto and Tanskanen, 1983; Khaw and Barret-Conner, 1984; Gruchow, Sobocinsky and Barboriak, 1985; Elliott *et al.*, 1987; Joffres, Reed and Yano, 1987) did not find any association between blood pressure and either dietary fat or cholesterol. Only one epidemiological study of Japanese immigrants living in Hawaii showed that blood pressure fell with an increase in total dietary fat and cholesterol (Reed *et al.*, 1985). The WHO CARDIAC cross-sectional study, which was conducted on a world-wide basis, demonstrated a significant positive correlation between serum cholesterol levels and diastolic blood pressure (Yamori *et al.*, 1993). Although there are methodological limitations in determining the effect of dietary fat on hypertension, the current evidence indicates that chronic elevation of plasma cholesterol is associated with higher diastolic blood pressure, probably as a result of atherosclerotic vascular changes.

Fatty acids

Saturated fat and monounsaturated fatty acids. Two dietary surveys carried out in Finland showed a significant inverse association between the intakes of saturated fat and blood pressure (Salonen, Tuomilehto and Tanskanen, 1983; Salonen *et al.*, 1988), however, many other studies found no such association (Gruchow, Sobocinski and Barboriak, 1985; Elliott *et al.*, 1987; Joffres, Reed and Yano, 1987; Williams *et al.*, 1987; Rubba *et al.*, 1987). In several populations studied, the level of saturated fatty acids in the adipose tissue tended to be inversely related to blood pressure (Riemersma *et al.*, 1986; Hudgins, Hirsch and Emken, 1991).

Controlled trials testing the effect of various dietary monounsaturated fatty acids showed no significant effects (Mensink, Janssen and Katan, 1988; McDonald *et al.*, 1989).

Polyunsaturated fatty acids. Regulation of blood pressure is impaired in animals which are deficient in linoleic acid. When such animals were made hypertensive by 9 days of drinking saline, the addition of linoleic acid to the diet normalized blood pressure despite the continued administration of saline (Cox *et al.*, 1982). When there is no deficiency, linoleic acid has little effect on blood pressure in animals (Smith-Barbaro *et al.*, 1980; McGregor, Morazain and Renaud, 1981; Mogenson and Box, 1982; Tobian *et al.*, 1982; Singer *et al.*, 1990; Shimamura and Wilson, 1991).

The results of human cross-sectional studies provide little evidence of an effect of n-6 fatty acid intake on blood pressure. The National Health and Nutrition Examination Surveys (NHANES) of adults in the USA indicated that the nutritional factor most strongly and consistently related to blood pressure was body mass index (Harlan *et al.*, 1984); serum calcium was related directly and serum phosphorus was related indirectly to systolic blood pressure. The study failed to show any relationship between diastolic blood pressure and dietary fats. In general, intakes of fatty acids and total fat, as determined by dietary histories, are not significantly correlated with blood pressure (Sacks, 1989). Moreover, there is little convincing evidence that the amount or type of dietary fat has an effect in persons with normal or mildly elevated blood pressure. Cross-sectional population studies are often confounded by the complexity of dietary and other life-style differences. The NI-HON-SAN study found that fat provided 15 percent of energy intake in Japan. Among ethnic Japanese in Hawaii and San Francisco, energy intakes from fats were 33 percent and 38 percent, respectively. The differences were mainly due to intakes of saturated fat (Kagan, Marmot and Kato, 1980). Blood cholesterol levels paralleled fat intake among all three groups. While blood pressures were similar in studies in Japan and Hawaii, they were higher in San Francisco. The prevalence of stroke, hypertension, hypertensive heart disease and left ventricular hypertrophy were higher in Japan than in the other two populations, possibly because of the higher intake of salt and alcohol and the lower intake of protein.

The ratio of polyunsaturated fat to saturated fat is generally higher in vegetarians than in non-vegetarians. The consumption of food of animal origin has been found to be highly correlated with both systolic and diastolic blood pressures (Sacks, Rosner and Kass, 1974). Vegetarian Seventh-Day Adventists in western Africa had lower blood pressures than non-vegetarians and showed a gradient of increasing blood pressure with increasing egg consumption (Armstrong, van Merwyk and Coates, 1977). With a lacto-ovo-vegetarian diet, normotensive persons showed a lowering of blood pressure (Rouse, Armstrong and Beilin, 1983). In line with this, a fall in systolic blood pressure in untreated, mildly hypertensive persons who changed to a vegetarian diet has been demonstrated (Margetts *et al.*, 1986). Replacing saturated fat with carbohydrate or with oils high in linoleic acid, however, does not always produce a favourable change in blood pressure (Sacks *et al.*, 1987). Many factors are influenced by vegetarian dietary habits, consequently the interpretation of studies from these populations is limited. It would be difficult to find an association of dietary fat with blood pressure, even if it exists, because of the low sensitivity of dietary methods such as the 24-hour recall and 3-4 day record of food frequency. However, biochemical analyses on the ratio of polyunsaturated to saturated fatty acids in adipose tissue also showed no association with blood pressure (Berry and Hirsch, 1986; Riemersma *et al.*, 1986; Ciocca *et al.*, 1987; Rubba *et al.*, 1987).

Seven studies have shown a significant decline in systolic blood pressure, up to 13 mm Hg, and diastolic blood pressure, 7 mm Hg, in mildly hypertensive individuals eating n-6 enriched diets (Iacono and Dougherty, 1993). Seven other studies were identified in which no significant change in blood pressure occurred in individuals eating similarly enriched diets. Differences in subject selection or adherence to diet may account for these observations. Five community-based intervention studies in Finland, Italy and the USA showed decreases in blood pressure with an increased ratio of intake of n-6 polyunsaturated fat to saturated fat. Two extensive, prospective cohort studies of health professionals in the USA, one covering females (Witteman *et al.*, 1989) and the other involving males (Ascherio *et al.*, 1992) did not show any association between dietary polyunsaturated fatty acids and the development of hypertension during a period of 4 years. In two, large, controlled intervention trials, conducted by the National Diet Heart Study Research Group (1968) and the Research Committee to the Medical Research Council (1968), no significant influence of dietary fat on blood pressure was observed in normotensives.

N-3 fatty acids. Many experimental studies have examined the effect of the n-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The discrepancies in observations on blood pressure may be due to the complexity of regulatory mechanisms. The syntheses of vasodilator prostaglandins, such as prostacyclin and PGE_2, as well as constrictors such as thromboxane A_2 and leukotriene B_2, are suppressed by the production of three series of eicosanoids. Thromboxane A_3 from n-3 fatty acids is not as active as the 2-series of eicosanoids. The effect of n-3 fatty acids on blood pressure is, therefore, due to the balance of vasodilator and vasoconstrictor eicosanoids in the vascular wall and in the kidney (Yin, Chu and Beilin, 1992; Shimokawa *et al.*, 1987; Lorenz *et al.*, 1983; Beilin, 1992).

In a controlled trial, 50 ml of fish oil (15g of n-3 fatty acids) decreased systolic and diastolic blood pressure in mildly hypertensive subjects, but 10 ml of fish oil was ineffective (Knapp and Fitzgerald, 1989). Supplementation of a diet with EPA plus DHA, compared to linoleic acid or α-linolenic acid, also lowered blood pressure (Kestin *et al.*, 1990).

When the consumption of fish (100 g of mackerel per day) was compared to meat, there was no effect on blood pressure and bleeding times were significantly prolonged (Houwelingen *et al.*, 1987). A trial in which there was supplementation of either oil containing EPA + DHA or the same amount of corn oil indicated that a reduction in blood pressure depended on the increase in plasma phospholipid n-3 fatty acids (Bonaa *et al.*, 1990).

In the elderly, a reduction in blood pressure occurred with fish oil only when it was combined with a low intake of sodium (Cobiac *et al.*, 1992). It may be noted that there was a high incidence of hypertension and haemorrhagic stroke among the Japanese who ate fish with salt.

The WHO multi-centre, cross-sectional study, known as CARDIAC, involving 55 centres in 24 countries, showed that serum cholesterol levels were positively related to diastolic blood pressure in populations worldwide (Yamori *et al.*, 1992).

For effective treatment of hypertension, intakes of n-3 fatty acids from foods would generally be too high for practical use. Such use should be based on their potential benefits for preventing atherosclerotic or thrombotic disease.

Conclusion

Modification of dietary fat to lower blood lipids indirectly affects blood pressure by slowing down or reversing the atherosclerotic process. Although higher levels of n-6 and long chain n-3 fatty acids lower elevated blood pressure, their effect is modest, especially when compared to the effects of weight reduction or sodium restriction.

Chapter 14

Non-glyceride constituents of fats

In addition to triacylglycerols, a variety of components are found in dietary fats which are important in maintaining health. These non-glyceride constituents of fats may explain some of the inconsistencies in epidemiologic and experimental studies.

Fat-soluble vitamins

Vitamin A and vitamin D occur prominently in butterfat and fish oils. Red palm oil is a potent source of ß-carotene, a provitamin A. In many developing countries, especially in West Africa, crude palm oil is an important source of ß-carotene, providing much of the vitamin A which is required by the populations.

The processing of edible oils, however, often results in the total removal of the carotenoids present in the crude oil. For example, crude palm oil, a rich source of carotenoids (500-700 ppm) may lose all of its carotenoids in the refining process.
However, it is possible to use mild techniques for processing crude palm oil that result in the retention of a major portion of the carotenoids while removing the undesirable free fatty acids and peroxides of the crude oil. The resulting red palm oil, with its high carotenoid content, could become an important dietary component in the battle against vitamin A deficiency in many developing countries and its use should be encouraged.

Many vegetable oils and the products made from them, contain appreciable concentrations of vitamin E (tocopherols) which may be reduced by some processing methods as well.

Ubiquinones

It is not known if ubiquinone Q_9 is biologically active in humans, but ubiquinone Q_{10} is active as a mitochondrial electron carrier. Ubiquinone$_{10}$, together with α-tocopherol, appears to protect low density lipoproteins from oxidation (Tribble *et al.*, 1994).

Antioxidants

Substances other than vitamin E act as antioxidants, but tocopherol is the principal fat-soluble antioxidant in the body and is found in lipoproteins, especially LDL. It is found within membranes both inside and outside cells, enhancing the cell's protection against free radical attack. Vitamin E enhances immune function (Meydani *et al.*, 1990) and can play a role in the repair of damaged membranes (Newmark and Mergens, 1981; Bright-See and Newmark, 1983).

It has been hypothesized that dietary antioxidants protect against a variety of age-related conditions, including cardiovascular disease and cancer. The role of tocopherol and other antioxidants in protecting unsaturated fatty acids from oxidation gained attention with the recognition that oxidized LDL could be involved in the atherosclerotic process (Jurgens *et al.*, 1987). Oxidatively modified LDL occurs in the atherosclerotic lesion and appears to be the link between plasma LDL and the development of early lesions (Yla-Herttuala *et al.*, 1989). Further evidence that lipid oxidation could be a factor in atherosclerosis came from the finding that the susceptibility of LDL to oxidation was correlated with the severity of atherosclerosis in young male survivors of myocardial infarction (Regnstrom *et al.*, 1992).

Antioxidant supplementation of men with low antioxidant status and a high intake of fat lessened the capacity of platelets to aggregate and to produce thromboxane A_2 (Salonen *et al.*, 1991). In line with this finding, stored serum samples of 16 European groups provided evidence of an inverse relationship between plasma α-tocopherol levels and mortality rates from coronary heart disease (Gey *et al.*, 1991). When a cohort of male volunteers received supplementation with α-tocopherol, compared to a placebo, during a 3 month period, this led to enhanced plasma and LDL α-tocopherol levels and decreased susceptibility of LDL to oxidation (Jialal and Grundy, 1992). In two large prospective studies, one among women (Stampfer *et al.*, 1993) and another among men (Rimm *et al.*, 1993), intake of vitamin E, primarily in the form of supplements, was associated with a substantially reduced risk of myocardial infarction. Among men, intake of dietary carotenoids was associated with lower risk as well, especially among individuals who had smoked cigarettes prior to or during the study. Current evidence suggests that antioxidants play an important role in the prevention of coronary heart disease, but further scientific support is required.

Fat-soluble antioxidants, particularly carotenoids and tocopherol, have been hypothesized to reduce the risk of various cancers. The best evidence indicates a relationship between carotenoid intake and risk of epithelial malignancies, particularly lung cancer (Steinmetz and Potter, 1991). Relatively few data exist relating vitamin E intake to the risk of cancer, in part because of the difficulty of assessing intakes of this nutrient. Nevertheless, blood levels of vitamin E (Knekt *et al.*, 1988) and vitamin E supplements (Gridley *et al.*, 1992) have been positively related to lower risk of several cancers. Also, vitamin A, both preformed and from a carotenoid precursor, has been found to be inversely related to the risk of breast cancer (Hunter *et al.*, 1993).

There is evidence from animal and epidemiological studies that ß-carotene and possibly α-carotene, may have anti-cancer properties. Epidemiological studies have consistently shown associations between high intakes of ß-carotene-enriched foods with reduced risk of certain cancers (Connett *et al.*, 1989; Le Marchand *et al.*, 1989; Peto *et al.*, 1981).

Tocotrienols

Apart from their vitamin E activity, tocotrienols exhibit certain physiological properties not observed with the tocopherols. When administered in the diet of animals and humans, tocotrienol concentrates have been reported to manifest a hypocholesterolemic effect (Burger *et al.*, 1984; Qureshi *et al.*, 1991 a, b; Tan *et al.*, 1991). It has been suggested that the cholesterol-lowering potential of the tocotrienols may be mediated by their ability to decrease levels of hepatic HMG-CoA reductase activity (Qureshi *et al.*, 1986). In addition, tocotrienols have been shown to influence certain hemostatic parameters (Qureshi *et al.*, 1991 a) and to reduce the occurrence of chemical-induced tumours in rats (Tan and Chu, 1991; Gould *et al.*, 1991).

Phytosterols

Plant sterols are not well-absorbed by humans and may inhibit cholesterol and bile acid absorption. They can have appreciable effects on LDL cholesterol levels, even at relatively low intakes (Grundy and Mok, 1977; Lees *et al.*, 1977; Heinemann, Leiss and von Bergmann, 1986). While the principal mechanism of action of phytosterols has not been established, they can influence micellar cholesterol solubilization (Child and Kuksis, 1986) as well as the rate of cholesterol synthesis and degradation (Bober, Akerlund and Bjorkhem, 1989, Ikeda and Sugano, 1983; Heinemann, Leiss and von Bergmann, 1986; Heinemann *et al.*, 1991).

A group of ferulic acid esters of triterpene alcohols and plant sterols has shown hypocholesterolemic effects as well, perhaps by inhibiting cholesterol absorption and enhancing sterol and bile acid excretion (Nicolosi, Ausman and Hegsted, 1990, 1991 a, b). Oryzanol, a ferulic acid ester, constitutes as much as 20 percent of the non-saponifiable fraction of crude rice bran oil (Rogers *et al.*, 1993). In addition, ferulic acid is a potent antioxidant which stabilizes vegetable oils.

Conclusions

Current data on antioxidants, specifically tocopherol, suggest a protective effect against coronary heart disease in humans. However, these data do not yet warrant specific recommendations for their intake. The hypothesis that oxidized LDL is an important atherogenic factor is generally regarded as attractive. Although oxidized LDL has been detected in atherosclerotic plaque, solid evidence that lipoprotein oxidation in humans is causally related to atherosclerosis has not been reported yet.

A number of studies seem to support the hypothesis that antioxidants can prevent oxidative modification of LDL. However, before these studies can be extrapolated to atherosclerosis, several points should be considered: first, demonstrating a protective effect in humans is difficult because the available techniques are limited in their ability to measure ongoing lipid peroxidations; second, the implications of the measurement of LDL oxidation *in vitro* for the *in vivo* situation are not clear; and, third, the efficacy of the individual antioxidants in the prevention of atherosclerosis has yet to be established in large intervention studies.

Foods high in polyunsaturates should contain at least 0.6 mg tocopherol equivalents per gram of polyunsaturated fatty acids. Higher levels may be necessary for fats rich in fatty acids containing more than two double bonds.

The introduction of low-fat spreads and dressings reduces vitamin E intake. In light of the emerging evidence on the importance of vitamin E, it may be prudent to have the same amount of vitamin E per gram of product in such low-fat products as occur in corresponding high-fat products.

Chapter 15

Nutrition labelling

Information about the contents of foodstuffs should be available to consumers. Listing the ingredients is one way to identify the nature of the food consumed, while providing a label which explains the nutrient content of the item is another. Labelling foods with their lipid components has received considerable attention because of consumer demand and the number of countries which are currently recommending that the general population modify their intake of dietary fats. While the appropriateness of this strategy and the ability to use such information may vary by country, health status of individuals, public health goals, and dietary patterns of different population groups within countries, it is expected that the increased availability of nutrition labels on food products will improve public health.

Characteristics of the nutrition label. Nutrition labels describe the nutrient content of a food and are intended for the consumer. Therefore, the terminology used should be meaningful and understandable to the general public. A standardized and simple format is likely to assist consumers in using food labels and in comparing foods. The nutrition information provided must be selected on the basis of consistency with dietary recommendations. Selection of the specific nutrients or food components to be listed must take into account label space, the analytical feasibility of measuring the particular nutritional component within the food matrix, and the relative costs of such analyses.

Implementing nutrition labelling. It should be noted that nutrition labelling may be questionable or irrelevant in a number of circumstances. Specifically, the nutrition label may not be cost effective or appropriate where the primary public health concern is lack of sufficient food, where educational levels are inadequate to allow consumers to read or comprehend such information, and where packaging and distribution methods for foodstuffs preclude the use of labels on or near foods. Nonetheless, it is likely that many countries have some segments of the population that would benefit from information about the lipid components of foods. In these cases, countries should consider the need to provide for appropriate labelling and its presentation relative to existing guidelines and approaches.

As nutrition labelling efforts have evolved, different approaches and legal requirements have been established. These create difficulties in developing and harmonizing nutrition information listings which have broad international applications. This includes considerations such as the number of languages to be used on the label; whether information should be expressed quantitatively, with symbols, or using descriptive phrases such as "high," "medium" or "low"; and whether the nutrition information is expressed in amounts per 100 grams of food or per specified serving. As the scientific evidence continues to emerge linking the nutrient content of foods to certain chronic disease conditions, public health policy and dietary guidance may shift and vary from country to country. While such situations are often unavoidable, it is clear that greater consistency in approaches to nutrition labelling would result in fewer barriers to promoting international harmonization and in greater benefits for consumers.

Codex Alimentarius Commission. The Codex Alimentarius Commission (CAC) was established to implement the Joint FAO/WHO Food Standards Programme. The *Guidelines on Nutrition Labelling* created by Codex are based on the principle that no food should be described or presented in a manner that is false, misleading or deceptive (Codex Alimentarius Commission, 1992). The guidelines include provisions for voluntary nutrient declaration, calculation and presentation of nutrient information. The *Guidelines on Claims* establish general principles to be followed and leave the definition of specific claims to national regulations (*Ibid.*). The CAC has indicated a need to address the specific issue of health and nutrition claims as they are commonly used. Current efforts are centered on developing a proposed draft on specific guidelines for claims.

Concerns

The nutrition information provided should be truthful and not mislead consumers. At the same time, labelling regulations should provide incentives to manufacturers to develop products that promote public health and assist consumers in following dietary recommendations. These concerns extend to the use of health- or nutrient content-related claims about a food's desirable attributes (such as "low in fat" or "cholesterol free") which are made to promote certain food products. In many circumstances, such claims can be helpful to consumers; however, claims may be problematic when they suggest that a particular brand of a food that is inherently "free" or "low" in fat has been specially formulated and has some benefit compared with other brands. Similarly, when changes in foods are developed or formulated to suggest benefits, these must be substantial. However, this will vary according to the food category.

All of these concerns are directed not only to statements made on the food label but also to advertising for the food products. As the capacity for rapid and extensive world-wide communication grows along with the development of global brands, advertising claims about the nutritional qualities of foods have the possibility of wide use. Consideration should be given to their regulation and procedures for enforcement of standards. Moreover, the concern about claims and their potential to mislead consumers extends to restaurants and food service operations. However, rules should not be so strict as to be a disincentive for food manufacturers or providers to formulate improved products and communicate information to the public.

Another fundamental concern is whether declarations for lipid components of food should be defined for labelling purposes by their chemical structures or their physiological endpoints. From the consumer education point of view, physiological endpoints have advantages because consumers may readily apply such information. However, the supporting data for linking certain lipid components within foods to specific physiological endpoints varies; some are well-established and accepted while others are suggestive and speculative. As an example, the interest in declaring the levels of "cholesterol-raising fatty acids" would be difficult to define at this time. Furthermore, there is no universal agreement about the appropriateness of providing consumers with relatively specific information about fatty acid components of foods. Undoubtedly, as the scientific evidence continues to accumulate, appropriate decisions in these areas will become clearer.

Conclusions

Efforts to increase both obligatory and voluntary nutrition labelling of foods in general, and the lipid components of foods in particular, should be supported. These general suggestions stem from the considerable evidence linking dietary fats to chronic diseases and the health recommendations concerning desirable levels of fat components in human diets.

Further, it is advantageous to pursue international harmonization in this area and the efforts of the Codex Alimentarius Commission, which is in the process of developing and establishing guidelines for nutrition labelling and food claims, should be supported. However, any efforts to harmonize details will be very complicated and these would be best accommodated in a small forum, for example, as an expert consultation or in *ad hoc* working groups.

There are specific recommendations for the appropriate components of the label. The total fat should be declared and should be defined as the sum of all fatty acids providing energy. This is consistent with the guidelines provided by Codex Alimentarius and the provisions of the European Community. Such declarations should be in gram increments. Saturated fat should be declared and should be defined as the sum of fatty acids without double bonds. This is also consistent with the guidelines provided by Codex Alimentarius and the provisions of the European Community. These declarations should be in gram increments as well. The cholesterol content should be declared in 10 milligram increments. Neither Codex Alimentarius nor the European Community have made provisions for the declaration of this fat component. Claims concerning saturated fatty acids in foods should be restricted to foods with appropriately low or limited levels of *trans* isomers of fatty acids. There is considerable agreement about the effects of *trans* fatty acids on serum cholesterol levels. It would be inappropriate to suggest an advantage of a food in reducing the risk of heart disease if it contained other components that clearly increase the risk of heart disease.

Although general agreement concerning appropriateness or desirability have not been reached, it may be useful to consider other special concerns relative to labelling. For instance, declarations for the caloric contribution of the fat content of the food. This should be as fat calories, not as percent fat. Declarations for the amount of *trans* isomers of fatty acids in a food, either as a separate listing or as a component of the saturated fat listing is another concern. Finally, declarations for the amount of n-3, n-6 and monounsaturated fatty acids should be considered.

Annex
National indices of dietary fat supplies

The following table provides information on national averages of the dietary energy and fat available for human consumption in 1988-90. The figures are based on FAO Food Balance Sheet data, and care needs to be taken to ensure that they are interpreted and used correctly. First, it should be stressed that they reflect overall averages of food and fat availability and do not represent actual dietary intakes. Second, it should be noted that the distribution and consumption of the food supplies, including dietary fat, available within a country is often highly skewed, usually along socio-economic lines and between urban and rural areas. This means, for example, that even in countries with low figures for total fat supply and low fat-energy ratios, many individuals and population groups will have normal or high fat intakes. Conversely, many people in countries with high indices of food and fat availability will not have access to an amount of food deemed adequate in this report.

COUNTRIES RANKED BY FAT ENERGY RATIO OF NATIONAL FOOD SUPPLY, 1988-90

(FAO Food Balance Sheets)

Country	FER[a] (%)	Total fat[b] (g)	Animal fat/ total fat[c] (%)	DES[d] (kcals)
RWANDA	7	15	23	1913
CAMBODIA	8	19	41	2122
BANGLADESH	9	20	15	2038
UGANDA	11	26	34	2177
VIET NAM	11	27	56	2216
KOREA DPR	11	36	40	2843
BURUNDI	12	25	13	1948
NEPAL	12	29	34	2205
MALAWI	12	28	16	2049
NIGER	12	31	23	2240
TANZANIA	12	31	29	2195
LAOS	13	34	58	2465
MADAGASCAR	13	32	52	2156
ZAMBIA	14	31	19	2016
ETHIOPIA	14	26	28	1699
ZAIRE	14	34	9	2130
MYANMAR	14	39	18	2454
LESOTHO	14	34	28	2121
MALDIVES	14	39	34	2400
PHILIPPINES	15	38	45	2341
INDIA	15	38	27	2229
YEMEN	15	38	32	2230
THAILAND	16	40	35	2280
CHINA	16	47	53	2645
PERU	16	37	47	2037
INDONESIA	16	47	11	2605
BOTSWANA	17	42	48	2260
GUATEMALA	17	42	20	2254
NICARAGUA	17	42	38	2235
MOROCCO	17	57	22	3031
GUYANA	17	47	41	2495
HAITI	17	38	22	2006
GHANA	17	41	12	2144
TOGO	17	43	14	2268
COMOROS	17	34	15	1760
SWAZILAND	18	52	33	2634
BENIN	18	47	13	2383
SRI LANKA	18	45	13	2246
AFGHANISTAN	18	35	39	1766
COTE D'IVOIRE	18	52	15	2569

a) fat energy ratio - the proportion of dietary energy supply derived from fat

b) average grams of fat available per caput per day

c) proportion of total fat supply derived from animal sources

d) Dietary energy supply - average total kilocalories available per caput per day

COUNTRIES RANKED BY FAT ENERGY RATIO OF NATIONAL FOOD SUPPLY, 1988-90

(FAO Food Balance Sheets)

Country	FER[a] (%)	Total fat[b] (g)	Animal fat/ total fat[c] (%)	DES[d] (kcals)
GUINEA	18	45	10	2242
BURKINA FASO	18	45	14	2219
GABON	18	49	29	2443
ANGOLA	18	38	31	1880
GAMBIA	18	47	15	2290
CAMEROON	19	46	20	2208
PAPUA N. GUINEA	19	53	33	2589
MOZAMBIQUE	19	37	9	1805
ALBANIA	19	54	59	2587
NIGERIA	19	47	7	2200
MALI	19	48	24	2259
KENYA	19	44	42	2064
CHAD	19	37	24	1735
SURINAME	19	52	40	2436
IRAN	19	66	28	3022
LIBERIA	20	49	10	2259
KOREA REP.	20	61	44	2826
NAMIBIA	20	44	44	1969
IRAQ	20	69	22	3096
DJIBOUTI	20	53	35	2362
MAURITIUS	20	65	33	2897
EGYPT	21	76	28	3310
BOLIVIA	21	46	62	2013
MAURITANIA	21	56	52	2447
CHILE	21	57	53	2484
EL SALVADOR	21	54	38	2331
CONGO	21	53	16	2295
ALGERIA	21	69	30	2944
COLOMBIA	21	58	47	2453
ZIMBABWE	21	53	22	2256
GUINEA-BISSAU	21	53	23	2235
SOUTH AFRICA	21	75	40	3133
PAKISTAN	22	57	34	2280
SEYCHELLES	23	59	33	2356
HONDURAS	23	56	30	2210
JORDAN	23	69	34	2711
TRINIDAD & TOB.	23	71	42	2770
SINGAPORE	23	79	71	3121
SOLOMON ISL.	23	58	25	2278
ST. VINCENT & GR.	23	63	41	2459
DOMINICAN REP.	23	60	31	2310
BRUNEI DARUSSALAM	24	75	54	2858

a) fat energy ratio - the proportion of dietary energy supply derived from fat

b) average grams of fat available per caput per day

c) proportion of total fat supply derived from animal sources

d) Dietary energy supply - average total kilocalories available per caput per day

COUNTRIES RANKED BY FAT ENERGY RATIO OF NATIONAL FOOD SUPPLY, 1988-90

(FAO Food Balance Sheets)

Country	FER[a] (%)	Total fat[b] (g)	Animal fat/ total fat[c] (%)	DES[d] (kcals)
CUBA	24	83	58	3129
COSTA RICA	24	72	41	2711
JAMAICA	24	68	36	2558
SAUDI ARABIA	24	79	38	2930
TURKEY	24	87	19	3197
SENEGAL	24	63	20	2322
CENTR. AFR. REP.	25	51	28	1846
REUNION	25	85	50	3082
JAPAN	25	81	48	2921
PANAMA	25	63	48	2269
SYRIA	25	87	34	3122
PARAGUAY	25	76	48	2684
BRAZIL	26	78	39	2730
KIRIBATI	26	72	20	2516
TUNISIA	26	90	19	3122
BELIZE	26	74	71	2575
ROMANIA	26	90	58	3081
VENEZUELA	26	71	35	2555
DOMINICA	26	85	47	2911
MEXICO	27	93	45	3062
GUADELOUPE	27	84	57	2776
MARTINIQUE	27	84	52	2768
SIERRA LEONE	28	58	6	1899
LEBANON	28	97	31	3142
FIJI	28	86	35	2769
GRENADA	28	74	44	2400
SAINT LUCIA	28	75	58	2424
SUDAN	28	63	38	2043
TONGA	28	93	47	2967
USSR (FORMER)	28	106	67	3380
FR GUIANA	28	89	69	2804
NEW CALEDONIA	29	94	45	2909
U. A. EMIRATES	29	106	58	3285
BAHAMAS	29	91	64	2776
LIBYA	30	108	29	3291
NETH. ANTILLES	30	88	63	2681
YUGOSLAVIA	30	117	59	3545
MONGOLIA	30	78	90	2361
FR. POLYNESIA	30	92	54	2757
BULGARIA	31	126	59	3695
ECUADOR	31	82	30	2399
SOMALIA	31	64	60	1874

a) fat energy ratio - the proportion of dietary energy supply derived from fat
b) average grams of fat available per caput per day
c) proportion of total fat supply derived from animal sources
d) Dietary energy supply - average total kilocalories available per caput per day

COUNTRIES RANKED BY FAT ENERGY RATIO OF NATIONAL FOOD SUPPLY, 1988-90

(FAO Food Balance Sheets)

Country	FER[a] (%)	Total fat[b] (g)	Animal fat/ total fat[c] (%)	DES[d] (kcals)
MALAYSIA	31	93	28	2671
BARBADOS	31	111	60	3217
ARGENTINA	31	107	63	3068
URUGUAY	31	93	76	2668
MACAU	32	81	53	2294
KUWAIT	32	108	47	3044
POLAND	32	123	79	3426
GERMANY, NL	33	135	73	3710
ANTIGUA & BARBUDA	33	85	58	2307
SAMOA	33	97	36	2621
PORTUGAL	34	125	47	3342
CZECHOSLOVAKIA	34	135	70	3574
OCEANIA	34	121	69	3172
ICELAND	34	132	73	3473
MALTA	34	121	56	3169
ISRAEL	35	126	33	3220
HONG KONG	35	112	57	2860
AUSTRALIA	37	135	73	3302
ST. KITTS & NEVIS	37	99	34	2435
VANUATU	37	113	36	2737
HUNGARY	37	150	76	3608
FINLAND	37	127	77	3067
ITALY	37	146	46	3498
NORWAY	38	135	64	3221
NEW ZEALAND	38	146	79	3460
GREECE	38	160	46	3775
CANADA	38	137	59	3242
USA	38	154	52	3642
GERMANY, FR	38	147	69	3472
SWEDEN	38	127	67	2977
BERMUDA	39	127	59	2960
UK	39	142	68	3270
IRELAND	39	173	66	3951
NETHERLANDS	40	135	60	3078
SÅO TOME PRN	40	95	5	2153
AUSTRIA	41	159	62	3486
FRANCE	42	168	66	3593
SPAIN	42	164	55	3473
SWITZERLAND	44	171	65	3508
BELGIUM-LUXEMBOURG	44	192	73	3925
DENMARK	46	185	80	3639

a) fat energy ratio - the proportion of dietary energy supply derived from fat

b) average grams of fat available per caput per day

c) proportion of total fat supply derived from animal sources

d) Dietary energy supply - average total kilocalories available per caput per day

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In their efforts to improve nutrition, the Food and Agriculture Organization of the United Nations and the World Health Organization convene expert consultations to provide advice to developing and developed countries. *Fats and oils in human nutrition* reviews the most recent scientific information on this crucial topic and presents the recommendations of the joint expert consultation held in Rome from 19 to 26 October 1993. Key factors that may influence consumption, health, food production and processing, food marketing and nutrition education are discussed. The report contains recommendations about desirable minimum and maximum intakes of fats; maternal and infant nutrition; essential fatty acids; saturated, unsaturated and isomeric fatty acids; antioxidants; and scientific and programmatic needs. An extensive bibliography is included.

ISBN 92-5-103621-7 ISSN 0254-4725
9 789251 036211
M-80 V4700E/2/12 98/2000